# EL406™

## Operator's Manual


BioTek®

# EL406™

## Microplate Washer Dispenser
## Operator's Manual

April 2009

Part Number 7181000
Revision B
BioTek® Instruments, Inc.

# Notices

**BioTek® Instruments, Inc.**

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Winooski, Vermont 05404-0998 USA

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# Contents

# Contacting BioTek Instruments, Inc.

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Winooski, Vermont 05404-0998 USA

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| | |
|---|---|
| Internet: | www.biotek.com |
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| E-Mail: | customercare@biotek.com |

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# Document Conventions

The intent of this Operator's Manual is to instruct the new user how to set up and operate BioTek's EL406™ Microplate Washer Dispenser. To help you read and understand this manual, certain document conventions have been used.

Major topic headings start a new page (such as **Document Conventions**, above) to give you a visual and style clue that a new major subject is being introduced. One or more subheadings may appear below each major heading.

This manual uses the following typographic conventions:

| Example | Description |
|---|---|
|  | These icons call attention to important safety notes. |
| **Warning!** | A **Warning** indicates the potential for bodily harm and tells you how to avoid the problem. |
| **Caution** | A **Caution** indicates potential damage to the instrument and tells you how to avoid the problem. |
|  | This icon calls attention to important information. |

**The menu path to open the feature described is shown in this format.**

When applicable, the above format shows you how to navigate to the feature described in that section.

❖ **Note:** Information of particular importance or usefulness is presented in this format.

 Tips or other helpful information is presented in this format.

# Revision History

| Rev | Date | Changes |
|---|---|---|
| A | 10/2008 | First Issue |
| B | 4/2009 | Full keypad control was added to EL406 and documented in this manual. Chapter 3, Operations includes complete instructions for operating the instrument using the keypad. |
| | | 1536-well plate processing capability is available with required hardware. Operating instructions and physical descriptions were updated where applicable throughout the manual. |
| | | New 1536-well hardware includes 128-tube aspirate manifold and two 32-tube Syringe dispenser manifolds. Installation instructions, Specifications, operation and maintenance sections were updated accordingly. |
| | | AutoPrime functionality was changed:<br>• Submerge tips, also known as soaking, capability was added;<br>• First-use control to trigger function was removed. AutoPrime, when enabled, always runs regardless of whether the device has been used since startup. |
| | | |

# Intended Use Statement

- The EL406™ Microplate Washer Dispenser provides microplate priming, washing, dispensing, and aspiration for ELISA, fluorescence and chemiluminescence immunoassays, cellular and agglutination assays.
- The EL406 can operate as a stand-alone instrument or with standard robotic systems, such as BioTek's BioStack™ Microplate Stacker.
- The intended use of this instrument is dependent on the instrument's labeling. If there is an IVD label, then the instrument may be used for clinical, research and development, or other non-clinical purposes. If there is no such label, then the instrument may **only** be used for research and development, or for other non-clinical purposes.

# Quality Control

It is considered good laboratory practice to run laboratory samples according to instructions and specific recommendations included in the assay package insert for the test to be conducted. Failure to conduct Quality Control checks could result in erroneous test data.

# Warranty & Product Registration

Please take a moment to review the **Warranty** information that shipped with your product. Please also **register** your product with BioTek to ensure that you receive important information and updates about the product(s) you have purchased.

You can register online through BioTek's Customer Resource Center (CRC) at www.biotek.com or by calling 888/451-5171 or 802/655-4740.

# Warnings

Use the EL406™ Microplate Washer Dispenser on a flat surface and away from excessive humidity.

When operated in a safe environment, according to the instructions in this manual, there are no known hazards associated with the EL406. However, the operator should be aware of certain situations that could result in serious injury:

**Do not reach** into the instrument during operation, as the washer manifold or peristaltic pump (Peri-pump) pump barrel may pinch your fingers. Do not reach for the microplate carrier until it is in its home position.

**Do not run** the Peri-pump without a cassette installed on the pump.

**Strict adherence** to instrument maintenance and qualification procedures is required to ensure accurate dispense volumes and risk-free operation.

# Hazards and Precautions

## Hazards

**Warning! Power Rating.** The EL406 must be connected to a power receptacle that provides voltage and current within the specified rating for the system. Use of an incompatible power receptacle may produce electrical shock and fire hazards.

**Warning! Electrical Grounding.** Never use a two-prong plug adapter to connect primary power to the EL406. Use of a two-prong adapter disconnects the utility ground, creating a severe shock hazard. Always connect the system power cord directly to a three-prong receptacle with a functional ground.

**Warning! Internal Voltage.** Always turn off the power switch and unplug the EL406 before cleaning the outer surface of the instrument.

**Warning! Liquids.** Avoid spilling liquids: fluid seepage into internal components creates a potential shock hazard. Wipe up all spills immediately. Do not operate the instrument if internal components have been exposed to fluid.

**Warning! Potential Biohazards.** Some assays or specimens may pose a biohazard. Adequate safety precautions should be taken as outlined in the assay's package insert. Always wear safety glasses and appropriate protective equipment, such as chemically resistant rubber gloves and apron.

**Warning! Ultrasonic Energy.** Ultrasonic energy is present in the ultrasonic cleaner reservoir when AutoClean programs are running (Ultrasonic Advantage™ models only). Avoid putting your fingers in the bath. Ultrasonic energy can be destructive to human tissue.

**Warning! Software Quality Control.** The operator must follow the manufacturer's assay package insert when modifying software parameters and establishing wash methods.

**Warning! Unspecified Use.** Failure to operate this equipment according to the guidelines and safeguards specified in this manual could result in a hazardous condition.

## Precautions

**Caution: Service.** Only qualified technical personnel should perform troubleshooting and service procedures on internal components.

**Caution: Environmental Conditions.** Do not expose the instrument to temperature extremes. For proper operation, ambient temperatures should remain between 10°– 40°C (50° –104°F). Performance may be adversely affected if temperatures fluctuate above or below this range. Storage temperature limits are broader.

**Caution: Chemical Compatibility.** Some chemicals may cause irreparable damage to this instrument. The following chemicals have been deemed safe for use in this instrument: buffer solutions (such as PBS), saline, surfactants, deionized water, 70% ethyl, isopropyl, or methyl alcohol, 40% formaldehyde, and 20% sodium hydroxide. Other chemicals may cause severe damage to the instrument. Contact BioTek Instruments if you require further assistance. Refer to the ***Chemical Compatibility listing in Appendix C***.

Never use DMSO or other organic solvents in the Washer component. These chemicals may cause severe damage to the manifold. Use of wash buffers containing acetic acid is limited to washers upgraded with PN 68098 Teflon® valves. Contact BioTek for upgrade information and prior to using other questionable chemicals.

**Caution: Sodium Hypochlorite.** Do not expose any part of the instrument to sodium hypochlorite solution (bleach) for more than 20 minutes. Prolonged contact may damage the instrument surfaces. Be certain to rinse and thoroughly wipe all surfaces.

**Caution: Buffer Solution.** Although many precautions have been taken to ensure that the instrument is as corrosion-proof as possible, the EL406™ is not sealed and liquids can seep into sensitive components. Make sure that any spilled buffer solution is wiped off its surfaces. Continuous exposure to salt solution may corrode parts of the microplate carrier, movement rail, springs, and other hardware.

**Caution: Bovine Serum Albumin.** Solutions containing proteins, such as bovine serum albumin (BSA), will compromise the washer's performance over time unless a strict maintenance protocol is adhered to. Specific instructions regarding BSA are provided with the ***Preventive Maintenance*** guidelines.

**Caution: Shipping Bracket.** The manifold shipping bracket must be removed prior to operating the EL406, and reinstalled before repackaging the washer for shipment. Follow the Installation instructions.

**Caution: Waste Sensor Port.** The waste sensor port on the back of EL406 instruments is the same type as the 24-VDC power connector on some other BioTek products (like the BioStack™ Microplate Stacker). Do not plug an external 24-VDC power supply into the waste sensor port! This will permanently damage components of the PCB inside the EL406.

**Caution: High Flow Pump Installation.** DO NOT plug the High Flow vacuum pump cable into a wall outlet! Use the adapter provided with the pump to connect the pump to the accessory outlet on the back of the washer. Follow the Installation instructions.

**Caution: Warranty.** Failure to follow preventive maintenance protocols may void the warranty.

**Caution: Disposal.** This instrument contains printed circuit boards and wiring with lead solder. Dispose of the instrument according to Directive 2002/96/EC, "on waste electrical and electronic equipment (WEEE)."

**Caution: Electromagnetic Environment.** Per IEC 61326-2-6 it is the user's responsibility to ensure that a compatible electromagnetic environment for this instrument is provided and maintained in order that the device will perform as intended.

**Caution: Electromagnetic Compatibility.** Do not use this device in close proximity to sources of strong electromagnetic radiation (e.g., unshielded intentional RF sources), as these may interfere with the proper operation.

# CE Mark

| | |
|---|---|
| CE | ***Based on the testing described below and information contained herein, this instrument bears the CE mark*** |

## Directive 2004/108/EC: Electromagnetic Compatibility

### *Emissions - Class A*

### *Emissions - Class A*

The system has been type tested by an independent, accredited testing laboratory and found to meet the requirements of EN 61326-1 and EN 61326-2-6: Class A for Radiated Emissions and Line Conducted Emissions. Verification of compliance was conducted to the limits and methods of the following:

CISPR 16-1, EN 55011

This equipment has been designed and tested to CISPR 11 Class A. In a domestic environment it may cause radio interference, in which case, you may need to mitigate the interference.

### *Immunity*

The system has been type tested by an independent, accredited testing laboratory and found to meet the requirements of EN 61326-1:2003 and CISPR 16-2:1999 for Immunity. Verification of compliance was conducted to the limits and methods of the following:

**EN 61000-4-2 Electrostatic Discharge**

**EN 61000-4-3 Radiated EM Fields**

**EN 61000-4-4 Electrical Fast Transient/Burst**

**EN 61000-4-5 Surge Immunity**

**EN 61000-4-6 Conducted Disturbances**

**EN 61000-4-8 Power Frequency Magnetic Field Immunity Test**

**EN 61000-4-11 Voltage Dips, Short Interruptions and Variations**

## Directive 73/23/EEC: Low Voltage (Safety)

The system has been type tested by an independent testing laboratory and was found to meet the requirements of EC Directive 73/23/EEC for Low Voltage. Verification of compliance was conducted to the limits and methods of the following:

#### EN 61010-1 (2001) 2nd Edition

"Safety requirement for electrical equipment for measurement, control and laboratory use. Part 1, General requirements."

#### EN 61010-2-101 (2002)

"Particular requirements for in vitro diagnostic (IVD) medical equipment."

## Directive 2002/96/EC: Waste Electrical and Electronic Equipment

This instrument contains printed circuit boards and wiring with lead solder. Dispose of the instrument according to Directive 2002/96/EC, "on waste electrical and electronic equipment (WEEE)."

#### EN 50419:2005 Marking

## Directive 2002/95/EC: Reduction of Hazardous Substances (RoHS)

This instrument is exempt from RoHS requirement per Article 2, Category 9.

## Directive 98/79/EC: *In Vitro* Diagnostics (some models)

- Product registration with competent authorities
- Traceability to the U.S. National Institute of Standards and Technology (NIST):

  **Microplate Washers:** Dispense precision and average residual volume is traceable to NIST.

**EN 61010-2-101:** Particular requirements for *in vitro* diagnostic (IVD) medical equipment.

# Electromagnetic Interference and Susceptibility

## USA FCC CLASS A

**Warning:** Changes or modifications to this unit not expressly approved by the manufacturer could void the user's authority to operate the equipment.

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules.

These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. Like all similar equipment, this equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause interference, in which case the user will be required to correct the interference at their own expense.

## Canadian Department of Communications Class A

This digital apparatus does not exceed Class A limits for radio emissions from digital apparatus set out in the Radio Interference Regulations of the Canadian Department of Communications.

Le present appareil numerique n'met pas du bruits radioelectriques depassant les limites applicables aux appareils numerique de la Class A prescrites dans le Reglement sur le brouillage radioelectrique edicte par le ministere des Communications du Canada.

# User Safety

This device has been type tested by an independent laboratory and found to meet the requirements of the following:

- **Underwriters Laboratories UL 61010-1: 2004**

  "Safety requirements for electrical equipment for measurement, control and laboratory use; Part 1: general requirements"

- **Canadian Standards Association CAN/CSA C22.2 No. 61010-1-04**

  "Safety requirements for electrical equipment for measurement, control and laboratory use; Part 1: general requirements"

- **EN 61010 Standards – See CE Mark List**

# Safety Symbols

Some of these symbols may appear on the instrument.

**Alternating current**
Courant alternatif
Wechselstrom
Corriente Alterna
Corrente alternata



**Direct current**
Courant continu
Gleichstrom
Corriente continua
Corrente continua



**Both direct and alternating current**
Courant continu et courant alternatif
Gleich-und Wechselstrom
Corriente continua y corriente alterna
Corrente continua e corrente alternata

**Earth ground terminal**
Borne de terre
Erde (Betriebserde)
Borne de tierra
Terra (di funzionamento)

**Protective conductor terminal**
Borne de terre de protection
Schutzleiteranschluss
Borne de tierra de protección
Terra di protezione



**On (Supply)**
Marche (alimentation)
Ein (Verbindung mit dem Netz)
Conectado
Chiuso

**Off (Supply)**
Arrêt (alimentation)
Aus (Trennung vom Netz)
Desconectado
Aperto (sconnessione dalla rete di alimentazione)

**Warning, risk of electric shock**
Attention, risque de choc électrique
Gefährliche elektrische Schlag
Precaución, riesgo de sacudida eléctrica
Attenzione, rischio di scossa elettrica

**Caution (refer to accompanying documents)**
Attention (voir documents d'accompanement)
Achtung siehe Begleitpapiere
Atención (vease los documentos incluidos)
Attenzione, consultare la doc annessa

**Warning, risk of crushing or pinching**
Attention, risque d'écrasement et pincement
Warnen, Gefahr des Zerquetschens und Klemmen
Precaución, riesgo del machacamiento y sejeción
Attenzione, rischio di schiacciare ed intrappolarsi

**Consult instructions for use**
Consulter la notice d'emploi
Gebrauchsanweisung beachten
Consultar las instrucciones de uso
Consultare le istruzioni per uso


***In vitro* diagnostic medical device**
Dispositif médical de diagnostic *in vitro*
Medizinisches *In-Vitro*-Diagnostikum
Dispositivo médico de diagnóstico *in vitro*
Dispositivo medico diagnostico *in vitro*

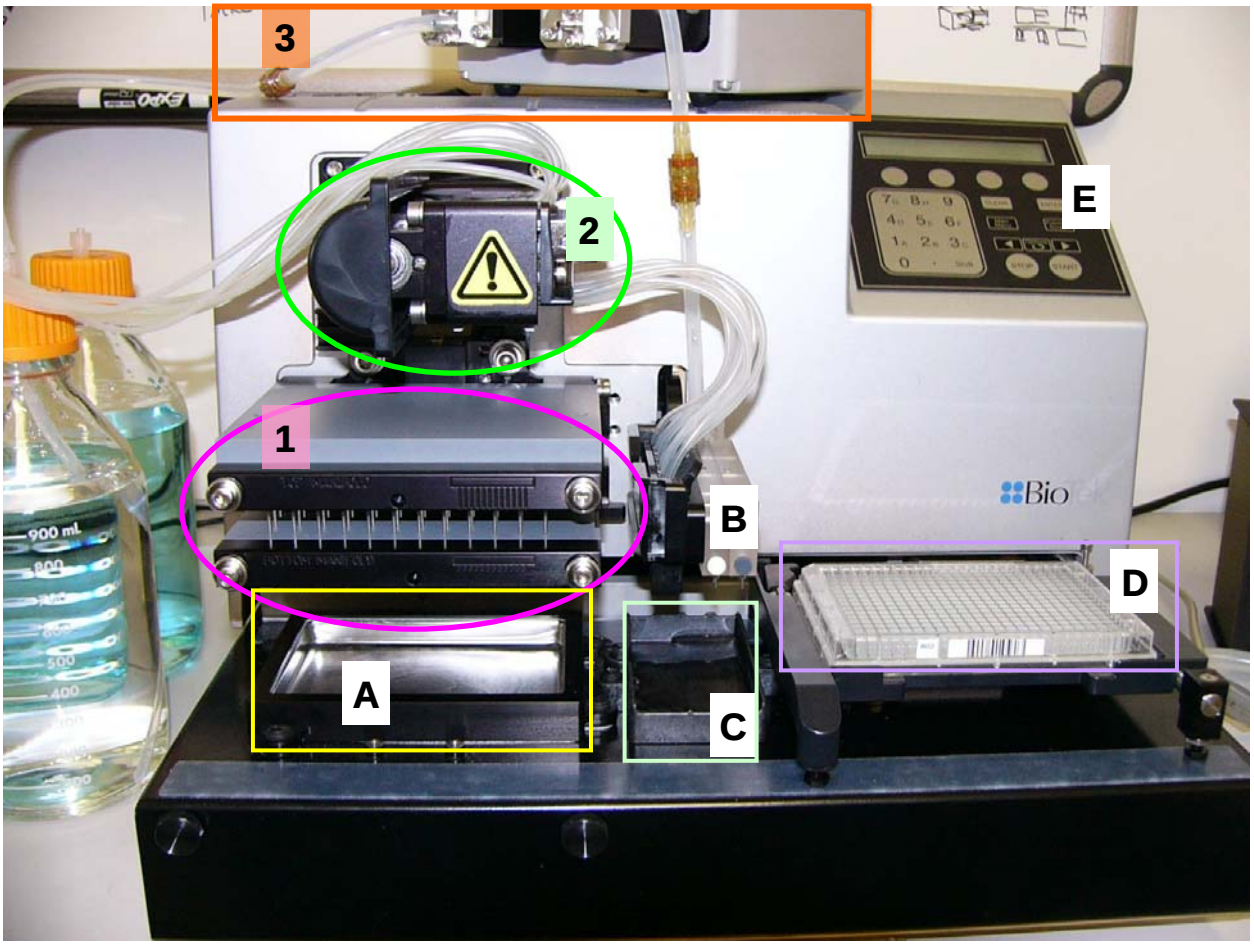
**Separate collection for electrical and electronic equipment**
Les équipements électriques et électroniques font l'objet d'une collecte sélective
Getrennte Sammlung von Elektro- und Elektronikgeräten
Recogida selectiva de aparatos eléctricos y electrónicos
Raccolta separata delle apparecchiature elettriche ed elettroniche

Chapter 1

# Introduction

This chapter introduces the EL406 Microplate Washer Dispenser.

# Introducing the EL406™




The EL406 offers two or three devices in one instrument: a microplate **Washer**, a peristaltic pump dispenser called the **Peri-pump**, and, optionally, a dual **Syringe dispenser** unit.

| | Device / Component | Description |
|---|---|---|
| 1 | **Washer manifold** | 96- or 192-tube devices aspirate and dispense fluid; or 128-tube manifold aspirates fluid from 1536-well plates. |
| 2 | **Peri-pump Dispenser** | Peristaltic, 8-channel dispenser with entirely visible fluid path. |
| 3 | **(Optional) Syringe Dispensers** | Two distinct syringe-pump dispensers, each with a 16- or 32-tube manifold. |
| A | **Ultrasonic cleaning and priming trough** | Supports AutoClean™ ultrasonic cleaning of the washer. |
| B | **Dispense heads** | Supports Peri-pump's Tip Holder and the Syringe dispensers' two manifolds. |
| C | **Priming trough** | Waste reservoir for collecting priming fluid for the Peri-pump and Syringe dispensers. |
| D | **Plate Carrier** | Holds standard microplates for processing. |
| E | **Keypad** | One of two ways to control the instrument; the other way is LHC. |

# Features of the EL406

- Supports all microplate-based assays, including ELISA, fluorescence, chemiluminescence, RIA, DNA probes, and cellular assays.
- A variety of solutions, including buffered saline and reagents can be dispensed.
- The intuitive menu-driven onboard software allows you to create and store up to 99 wash and dispense protocols. BioTek provides numerous pre-defined protocols for maintenance and instrument qualification purposes.
- Compatible with BioTek's BioStack™ Microplate Stacker and BioStack™ Twister II Microplate Handler for automated plate processing. See page 6, ***Compatibility with the BioStack System***.
- A robot-accessible carrier that can be interfaced into some robotic systems.
- Computer control using BioTek's **Liquid Handling Control™ software** ("LHC") and the **EL406 Interface Software**.
- A low-maintenance design, the result of BioTek's long history with liquid-handling instruments.

## Washer

- Programmable dispense volumes, flow, and aspiration rates for a wide range of wash options, from gentle washing for cellular assays to vigorous washing for ELISA™.
- A "bottom washing" routine to lower the background absorbance and "crosswise" or secondary aspiration to reduce residual volumes, except in 1536-well plates.
- Supports Wash, Prime, Dispense, and Aspirate steps (except dispensing to 1536-well plates, which is performed with the dispensers).
- An optional external buffer-switching module with four different supply bottles supports complex assays by allowing one protocol to automatically draw reagent from up to four separate reservoirs.
- Built-in fluid flow, fluid detection, and vacuum sensing provide complete protection for unattended operation.
- Several pre-defined programs are provided to simplify preventative maintenance, which should be performed regularly to ensure optimum washer performance.
- BioTek's patent-pending Ultrasonic Advantage™ (ultrasonic cleaner) provides extra cleaning power by using ultrasonic pulses in a water bath to remove residue on the manifold tubes. A stainless steel cleaning reservoir with an ultrasonic transducer bonded to the bottom of the reservoir is mounted on the washer. An **AutoClean** function enables the user to run ultrasonic cleaning routines.

## Peri-pump Dispenser

- A peristaltic pump with eight individual tubes transfers fluid from a supply bottle, or up to eight different supply bottles, to various vessels. The pump has four rollers over which the tubing is stretched.
- The tubing is contained in an easy to load and unload cassette that is attached to the pump head. The pump's protective cover must be in place to run a dispense routine.
- Three cassette sizes are available: 1 µL, 5 µL, and 10 µL for the most precise dispensing of volumes from 1 to 3000 µL.
- Autoclavable tubing (steam temperatures and pressures of 121° C and 1 bar (750 mmHg)) is compatible with 70% ethyl or isopropyl alcohol and 0.5% sodium hypochlorite (bleach) solution for easy maintenance.

## Syringe Dispensers

- The Syringe dispenser has a long-lasting seal that ensures precise and accurate fluid delivery, as well as reproducibility for repeated dispenses.
- Two syringes support distinct fluid sources, each with a 16- or 32-channel manifold to dispense to standard plates:
  - 16-channel: one tube per well for 384-well plates and two tubes per well for 96-well plates.
  - 32-channel: one tube per well for 1536-well plates.
- Autoclavable components can be used with organic solvents and provide easy maintenance.
- Does not require recalibration.

## Liquid Handling Control™ (LHC) Software

BioTek's Liquid Handling Control (LHC) software lets you control the EL406™ Washer Dispenser from your computer. You'll enjoy the convenience of programming assay-specific wash and dispense protocols in a familiar Windows environment (Microsoft® Windows Vista™, Windows XP, and Windows 2000). And, to keep the instrument in peak condition in busy laboratories, the LHC offers custom reminders that can be programmed for regular maintenance routines.

For high-throughput applications, the LHC supports BioStack™ integration.

Please refer to the ***LHC Installation Guide*** and ***Help system*** to learn about:

- **Installing** the LHC software on the controlling computer
- Running **Maintenance** protocols
- Enabling **AutoPrime**
- Running **Qualification** protocols
- Special considerations when operating with the **BioStack Microplate Stacker**

# Compatibility with the BioStack

The EL406 is compatible with BioTek's BioStack Microplate Stacker. The BioStack can rapidly transfer microplates one-at-a-time to and from the instrument, and includes:

- Removable stacks (one input and one output).
- Optional restacking of plates to maintain correct sequencing.
- The ability to continue processing plates following the aborting/failure of one plate.
- The ability to pause processing to allow the user to add more plates to the input stack or to remove some from the output stack.

If you have purchased the BioStack to operate with the EL406, refer to the BioStack Operator's Manual for instructions on configuring the EL406 to run with the BioStack. If you are interested in purchasing the BioStack, contact your local BioTek dealer for more information or visit our website at **www.biotek.com**.

# Package Contents

❖ Part numbers are subject to change over time. Contact BioTek Customer Care with any questions.

| Description | | PN |
|---|---|---|
| Power cord (part numbers vary according to country of use) | | Varies |
| RS-232 serial cable | | 75034 |
| USB cable (USB Virtual COM Port Driver Software & instructions) | | 75108 |
| Microplate carrier | | 7180501 |
| Priming trough insert (1) for Peri-pump | | 7182043 |
| Priming trough insert (2) for Syringe dispensers | | 7182044 |
| Mist shield and thumbscrews (2 *installed*) | | 7182042 |
| Strip plate (12x1) | | 98265 |
| Screwdriver, Phillips | | 98268 |
| Stylus – for cleaning 96- and 192-tube aspirate manifold tubes | | 7102108 |
| Stylus – for cleaning 96-tube dispense manifold tubes and 128-tube aspirate manifold tubes | | 2872304 |
| Stylus – for cleaning 192-tube dispense manifold tubes | | 7102139 |
| 10 cc Syringe and tubing for Peri-pump cassette maintenance | | 49919 |
| Shipping brackets (2) | | 7182005 |
| | | 7182073 |
| Instructions for removal of shipping brackets | | 7181003 |
| Hex wrench – 9/64” for removing washer manifold | | 48434 |
| Hex wrench – 3/32” for removing syringe pumps | | 48570 |
| Hex wrench – 1/16” for removing magnets from dispense manifolds | | 48713 |
| Spare fuses (3) | | 46055 |
| EL406 Installation Guide (and Operator’s Manual on CD - PN 7181009) | | 7181010 |
| EL406 Keypad Quick Guide | | 7181022 |
| Packing instructions | | 7181004 |
| Document kit: Warranty statement, Calibration certificate | | 94075 |
| Declaration of Conformity | For clinical use (IVD label) | 7181007 |
| Vacuum line filter | | 48294 |

# Optional Accessories

❖ Part numbers are subject to change over time. Contact BioTek Customer Care with any questions.

| Module | Description | PN |
|---|---|---|
| **Washer** | | |
| | Buffer Switching external valve module w/ 4 supply bottles | 7100540 |
| | Power cable for valve module | 7100503 |
| | Additional washer manifold: | |
| | 192-tube wash manifold (for HT 384-well plates) | |
| | 1536-well hardware: 128-tube aspirate manifold and 32-tube Syringe dispenser manifolds (2) | |
| | 4L bottles, standard vacuum pump: | |
| | Waste System (includes pump) 115V | 7100529 |
| | Waste System (includes pump) 230V | 7100546 |
| | 4L bottles, high flow vacuum pump: | |
| | Waste System (includes high flow pump) 115V | 7100584 |
| | Waste System (includes high flow pump) 230V | 7100585 |
| | 10L bottles, standard vacuum pump: | |
| | Vacuum pump, 115V | 7100562 |
| | Vacuum pump, 230V | 7100561 |
| | Waste System | 7100582 |
| | 10L bottles, high flow vacuum pump: | |
| | Waste System | 7100582 |
| | High flow vacuum pump 115V | 7100563 |
| | High flow vacuum pump 230V | 7100564 |
| | **Note: High flow pump recommended for 384-well washing with buffers not containing surfactant.** | |
| | | |
| **Peri-pump Dispenser** | | |
| | Cassettes for peristaltic dispenser | |
| | 1 µL Cassette – molded plastic tips | 7170012 |
| | 1 µL Cassette – sapphire jeweled tips | 7170015 |
| | 1 µL Cassette for 1536–well, sapphire jeweled tips | 7170016 |
| | 1 µL Cassette for 1536–well, molded plastic tips | 7170018 |
| | 5 µL Cassette – molded plastic tips | 7170011 |
| | 5 µL Cassette – stainless steel tips | 7170014 |
| | 10 µL Cassette – molded plastic tips | 7170010 |
| | 10 µL Cassette – stainless steel tips | 7170013 |
| | Replacement Cassette Tubing | |
| | 1 µL Replacement Tubing Set (molded plastic tips*) | 7170009 |
| | 5 µL Replacement Tubing Set (molded plastic tips*) | 7170008 |
| | 10 µL Replacement Tubing Set (molded plastic tips*) | 7170007 |

| | | |
|---|---|---|
| | Cassette Calibration Kit | 7170017 |
| | *Save your jeweled and steel tips for reuse with a replacement kit. | |

| | | | |
|---|---|---|---|
| **Syringe Dispensers** | | | |
| | Syringe Dispenser Module Kit | | 7180006 |
| | 2 32-Tube Dispense Manifolds (ordered with 1536-Well Plate Washer kit) | | |
| | Stylus – for cleaning 16-tube dispense manifold tubes | | 2872304 |
| | Stylus – for cleaning 32-tube LB dispense manifold tubes | | 7182095 |
| | Stylus – for cleaning 32-tube SB dispense manifold tubes | | 7182102 |
| | Inline Filters (2) | | 48705 |
| **General** | | | |
| | Liquid Handling Control™ Software | | LHC |
| | EL406™ Interface Software | | 7180202 |
| | EL406 Qualification Package (IQ-OQ-PQ) | | 7180527 |
| | BioStack Microplate Stacker | | BIOSTACK2WR<br>BIOSTACK2 |
| | BioStack Integration Kit for EL406 | | 7310027 |
| | Blue Test Dye Solution | For evacuation and dispense tests | 7773001 |
| | Wetting Agent Solution | | 7773002 |
| | | | |

# Hardware Specifications

| Labware | |
|---|---|
| Microplates: | 96-well, 384-well, 1536-well that comply with SBS microplate standards 1-2004, 2-2004, 3-2004, and 4-2004. |
| Microstrips: | 1 x 8, 1 x 12. |
| Microwells: | Flat, round, "V" bottom. |

| Hardware & Environmental | |
|---|---|
| **User Interface:** | 2-line x 24 character LCD screen<br>25 alphanumeric soft keys |
| **Power Supply:** | The instrument uses two internal power supplies: 24-volt 60 watt and 48-volt 60 watt. These supplies are compatible with 100-240 V~; 50-60 Hz. |
| Accessory Outlet: | ≤ 5.0 A, used for vacuum pump |
| **Dimensions:** | 16½ x 125/8 x 18 inches (42 cm x 32 cm x 46 cm) (WxDxH), |
| **Weight:** | 32 lb (14.5 kg) |
| **Operating Conditions:** | 10° - 40°C (50° - 104°F) |
| **Relative Humidity:** | The instrument should be operated in a non-condensing humid environment having a maximum relative humidity of 80% at temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C. |

## Washer

| Manifold Type | |
|---|---|
| 96-tube: | Dual manifold with 96 sets of aspirate and dispense tubes arranged in an 8x12 array to process 96-well microplates. |
| 192-tube: Optional | Dual manifold with 192 aspirate tubes and 192 dispense tubes arranged in a 16x12 array to process 384-well plates. |
| 128-tube: | Aspirate-only manifold with 128-tubes in 32X4 array to process 1536-well plates. One dispense pin in the manifold fills the priming trough for maintenance procedures. |

| Other | |
|---|---|
| Waste bottle volume: | 4 or 10 liters, depending on the accessory package. |
| Supply bottle volume: | 3.7 liter (4) with Buffer Switching module or optional 10 liter bottles. |

## Peri-pump Dispenser

| | |
|---|---|
| **Peristaltic pump:** | Positive displacement peristaltic pump with 4 rollers that stretch the 8 tubes (one per channel) to deliver fluid |

| Cassette Types | Dispense range | Cassette Life | Dead Volume |
|---|---|---|---|
| **1 µL** | 1 – 3000 µL | 1000 384-well plates @ 5 µL/well | 1.2 mL |
| **5 µL** | 1 – 3000 µL | 1000 96-well plates @ 50 µL/well | 4.2 mL |
| **10 µL** | 1 – 3000 µL | 1000 96-well plates @ 100 µL/well | 7.4 mL |

## Syringe Dispensers

| | |
|---|---|
| **Manifold Type:** | 1x16-channel autoclavable manifold with replaceable stainless steel tubes. |
| | 1x32-channel manifold cannot be autoclaved, and does not support non-factory tube replacement; inline 90-micron filter included to minimize clogs. |

# Performance Specifications

## Washer

| Average Residual Volume (Evacuation Efficiency) | |
|---|---|
| 96-Tube Manifold | Average residual volume in the wells is ≤ 2 µL per well after a 3-cycle wash, when 300 µL of deionized water with 0.1% Tween 20®, or buffer equivalent, is dispensed per well into a Costar® 96-well flat-bottomed plate. The aspirate height adjustment is optimized for the plate prior to testing. |
| 192-Tube Manifold | Average residual volume in the wells is ≤ 2 µL per well after a 3-cycle wash, when 100 µL of deionized water with 0.1% Tween 20, or buffer equivalent, is dispensed per well into a Costar 384-well flat-bottomed plate. The aspirate height adjustment is optimized for the plate prior to testing. |
| 128-Tube Manifold | Average residual volume in the wells shall be ≤ 0.1 µL per well when 10 µL of deionized water with 0.1% Tween 20 solution is dispensed per well into a 1536 well flat bottomed Nunc® plate. |

| Dispense Accuracy | |
|---|---|
| 96-Tube Manifold | ± 5% when dispensing 300 µL per well of deionized water with 0.1% Tween 20, with FD&C #1 blue dye at a rate of 300 µL per well per second into a Costar 96-well flat-bottomed plate. The weight of the fluid dispensed shall be measured gravimetrically. |
| 192-Tube Manifold | ± 5% when dispensing 80 µL per well of deionized water with 0.1% Tween 20, with FD&C #1 blue dye at a rate of 102 µL per well per second into a Costar 384-well flat-bottomed plate. The weight of the fluid dispensed shall be measured gravimetrically. |

| Dispense Precision | |
|---|---|
| 96-Tube Manifold | ≤ 3.0% CV when dispensing 300 µL per well of deionized water with 0.1% Tween 20, with FD&C #1 blue dye at a rate of 300 µL per well per second into a Costar 96-well flat-bottomed plate. The absorbance of the solution is read at 630 nm and 450 nm reference. |
| 192-Tube Manifold | ≤ 4.0% CV when dispensing 80 µL per well of deionized water with 0.1% Tween 20, with FD&C #1 blue dye at a rate of 102 µL per well per second into a Costar 384-well flat-bottomed plate. The absorbance of the solution is read at 630 nm and 450 nm reference. |

## Peri-pump

| Dispense Precision and Accuracy | | |
|---|---|---|
| Cassette | **Precision** is measured for multiple dispenses per channel using clean deionized water with 0.1% Tween 20 with FD&C #1 blue dye. The absorbance of the solution shall be read at 630 nm and 450 nm reference. Specifications apply to volumes that are full unit increments for the cassette to which they apply. For example: the precision specification for a 10 µL cassette is valid at 10, 20, 30, …, 3000 µL. | **Accuracy** is measured gravimetrically when dispensing clean deionized water. Specifications apply to volumes that are full unit increments for the cassette to which they apply. For example: the accuracy specification for a 10 µL cassette is valid at 10, 20, 30, ... 3000 µL. |
| 1µL | < 10% CV @ 1 µL per well | ± 10% @ 1 µL per well |
| | < 5% CV @ 2 µL per well* | ± 5% @ 2 µL per well* |
| 5µL | < 5% CV @ 5 µL per well | ± 4% @ 5 µL per well |
| | < 2.5% CV @ 10 µL per well* | ± 2% @ 10 µL per well* |
| 10µL | < 4% CV @ 10 µL per well | ± 4% @ 10 µL per well |
| | < 2% CV @ 20 µL per well* | ± 2% @ 20 µL per well* |
| | * These specifications are for these dispense volumes and higher. | |

## Syringe

| Dispense Accuracy Performance | |
|---|---|
| The weight of the fluid dispensed shall be measured gravimetrically. | |
| 16-Tube | When dispensing room temperature deionized water: |
| | 384-Well Dispense<br>±1 µL for volumes of 5 to 100 µL, or ±1% for all other volumes;<br>96-Well Dispense<br>±2 µL for volumes of 10 to 200 µL, or ±1% for all other volumes. |
| 32-Tube | ± 5% when dispensing 6 µL/well of deionized water with approximately 15% ethanol and FD&C #1 blue dye at flow rate 3. |

| Dispense Precision Performance | |
|---|---|
| 16-Tube | ≤ 2% CV typical when dispensing 100 µL of deionized water with FD&C #1 blue or #5 yellow dye, at dual wavelength using the appropriate wavelength for the dye.<br>< 5% CV (typical precision at 20 µL per tube)<br>< 10% CV (typical precision at 5 µL per tube)<br>*Note: Factory-tested using dispense volumes of 80 µL and 20 µL.* |
| 32-Tube | < 12% CV when dispensing 6 µL per well of deionized water with approximately 15% ethanol and FD&C #1 blue dye. Absorbance shall be read at 630 nm and 450 nm reference. |

# Glossary

| | |
|---|---|
| **Pre-dispense** | Pre-dispense is a quick prime (over the priming trough) to normalize or wet the tips or tubes to correct for evaporation and other minor fluid loss. All dispense steps can be preceded by a pre-dispense, and generally this is recommended for precise, accurate dispensing. Also called a "tip prime." |
| **Prime** | Thoroughly wetting the tubing and removing all air bubbles is required to meet performance specifications. Priming flushes the tubing and disposes fluid into the device's priming trough. The EL406 uses the vacuum pump to empty the fluid into the waste containers. Fluid used to prime the washer cannot be retrieved, but the Peri-pump and Syringe dispensers ship with "priming trough inserts" that sit in the priming trough to capture expensive reagents. |
| **Purge** | The Peri-pump dispenser offers “Purge” capability to reclaim fluid in the tubing. Purge reverses the flow direction, pumping fluid back into the supply vessel. |
| **Soak** | After dispensing fluid to the plate, a soak step temporarily stops the washer, allowing the fluids to interact. It can be combined with a Shake step to better mix the fluids. Your assay may benefit from a soak period to draw unbound reagent from the solid phase. |
| **Submerge** | The washer provides the ability to soak the manifold tubes in fluid for cleaning purposes. This option is enabled as part of a Prime step. After the priming fluid is dispensed into the trough, the tubes are lowered into the fluid, where they remain for the specified duration. |
| **Bottom wash** | For extra vigorous plate washing, a Bottom Wash adds an initial dispense-aspirate sequence (wash cycle) to the specified number of cycles. Fluid is simultaneously dispensed and aspirated to create cleaning turbulence, and ends with a final dispense to fill the wells. |
| **Cell wash** | Certain options and parameters are offered for extra-gentle plate washing. Find “Cell Wash” in the LHC Help and Operations chapter. |

# Product Support & Service

A superior support staff backs all of BioTek's products. If your instrument or software ever fails to function perfectly, if you have questions about how to use or maintain them, or if you need to send an instrument to BioTek for service or repair, please contact our Technical Assistance Center (TAC).

Our Technical Assistance Center is open from 8:30 AM to 5:30 PM (EST), Monday through Friday, excluding standard U.S. holidays. You can send a fax or an e-mail any time.

**Phone:** (800) 242-4685 or (802) 655-4740 **Fax:** 802-654-0638

**E-Mail:** tac@biotek.com

You can also request technical assistance from our website: **www.biotek.com**

Please be prepared to provide the following information:

- Your name and company information, along with a daytime phone or fax number, and/or an e-mail address
- The product name, model, and serial number
- The software configuration information: in the Liquid Handling Control™ (LHC) Software, select **Tools>Instrument Utilities**
- For troubleshooting assistance or instruments needing repair, the specific steps that produce your problem and any error codes displayed on the screen (see also ***Appendix A, Error Codes***).

## Returning Instruments for Service/Repair

If you need to return an instrument to BioTek for service or repair, please contact the TAC for a Return Materials Authorization (RMA) number *before* shipping the instrument. Repackage the instrument properly (see ***Chapter 2, Installation***), write the RMA number on the shipping box, and ship to this address:

**BioTek Instruments, Inc.**
ATTN: RMA# xxxxx
Highland Park
100 Tigan Street
Winooski, Vermont 05404 USA

## Contacting BioTek for Applications Support

BioTek's fully equipped Application Laboratory provides our on-staff scientists with the means to assist you with the integration of our instrumentation and software with your unique scientific applications.

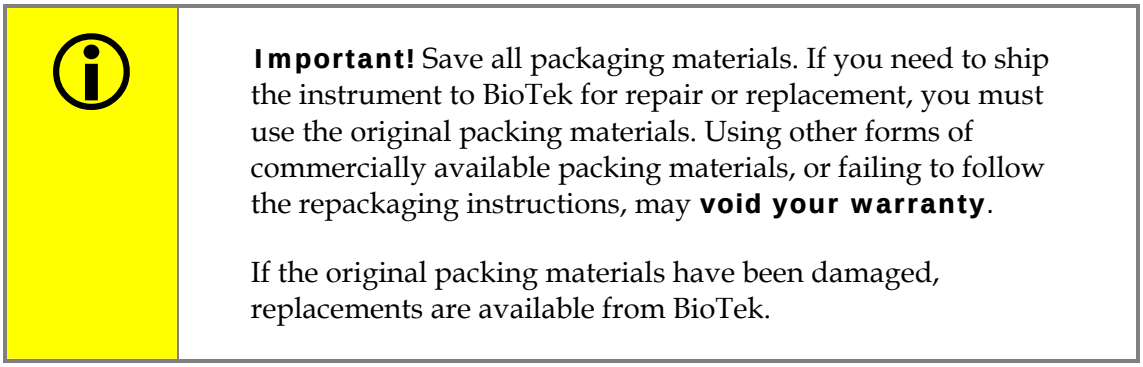
**Phone:** (888) 451-5171 **E-Mail:** applications@biotek.com

Chapter 2

# Installation

This chapter includes instructions for setting up the EL406™ Washer Dispenser and installing its components. Instructions for repackaging the instrument for shipment are also included.

# 1: Unpack and Inspect the Instrument



**Important!** Save all packaging materials. If you need to ship the instrument to BioTek for repair or replacement, you must use the original packing materials. Using other forms of commercially available packing materials, or failing to follow the repackaging instructions, may **void your warranty**.

If the original packing materials have been damaged, replacements are available from BioTek.

Inspect the shipping box, packaging, instrument, and accessories for signs of damage.

If the EL406 Microplate Washer Dispenser is damaged, notify the carrier and your BioTek representative. Keep the shipping cartons and packing material for the carrier's inspection. BioTek will arrange for repair or replacement of your instrument immediately, before the shipping-related claim is settled.

Unpack the boxes containing the instrument and other equipment:

- EL406 Microplate Washer Dispenser and accessories
- Buffer switching valve module and accessories
- Vacuum Pump and accessories
- Dual Syringe Dispensers and accessories
- Additional 192-tube washer manifolds for 384-well plate processing or 128-tube wash and 32-tube dispense manifolds for 1536-well plate processing.

## Accessories Checklist

Use this checklist to make sure you have all the accessories:

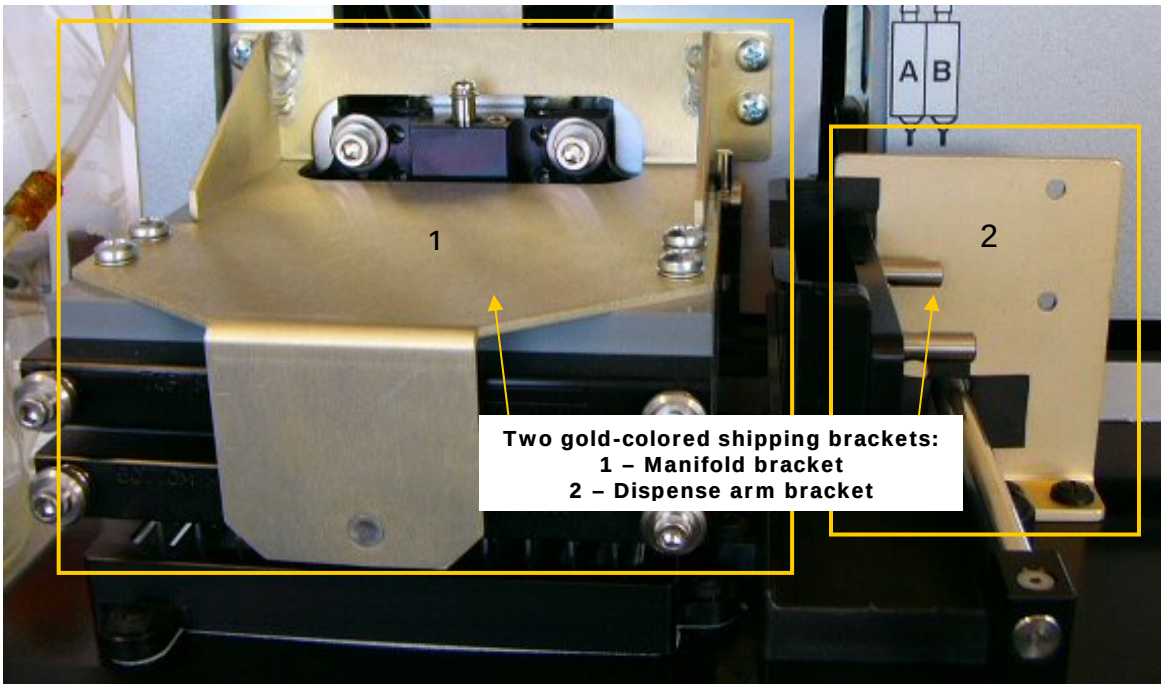
- ☐ 2 Peri-pump Dispense Cassettes (customer specified)
- ☐ Installation Guide and User's Manual on CD
- ☐ Microplate carrier
- ☐ Mist shield
- ☐ Strip plate 12 x 1
- ☐ Priming trough inserts (3)
- ☐ USB cable, USB Virtual COM Driver Software & instructions
- ☐ Power supply and power cord
- ☐ Serial cable (RS-232)

- ☐ Shipped in small plastic pouch:
- ☐ Screwdriver for calibrating cassettes
- ☐ 10 cc syringe and tubing for flushing cassette tubes and tips
- ☐ Velcro® strips for attaching plastic pouch on rear panel
- ☐ Hex wrenches (3)
- ☐ Spare fuses (3) for vacuum pump
- ☐ Stylus for cleaning manifold tubes, one for each manifold
- ☐ Buffer Switching (external valve) module
- ☐ Vacuum pump and accessories (various models)
- ☐ Bottles and tubing sets (various models)

**Optional accessories shipped separately:**

- ☐ Dual Syringe Dispenser module
- ☐ EL406™ Qualification and Maintenance Package
- ☐ Liquid Handling Control™ (LHC) software
- ☐ EL406™ Interface Software
- ☐ BioStack Integration Kit for EL406
- ☐ Cassette Calibration kit
- ☐ Additional manifold:
  - ☐ 192-tube wash manifold for processing 384-well plates
  - ☐ 128-tube aspirate and 32-tube dispense manifolds for processing 1536-well plates
  - ☐ Various accessories like stylus for cleaning tubes, inline filters (2)

# 2: Remove the Shipping Hardware




## Supplies

A Phillips-head screwdriver is needed to remove the shipping brackets.

Keep in mind that you will have to reinstall the shipping hardware and use the original shipping material if it is necessary to return the instrument to BioTek for service or repair.

## Remove the rubber band

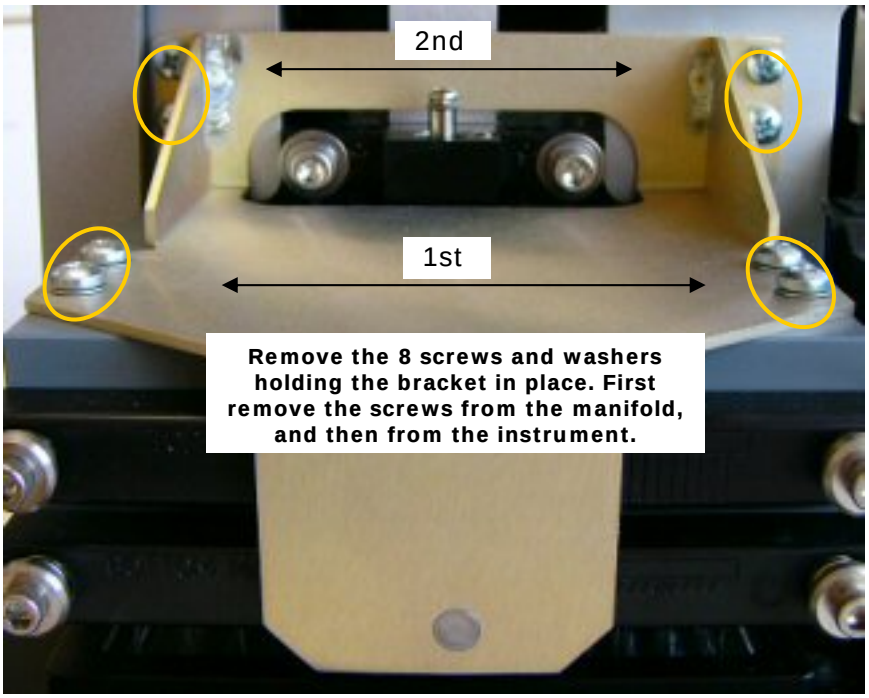
1. Carefully remove the rubber band that holds the pump cover in place around the Peri-pump dispenser.

   Store the rubber band in the small plastic storage pouch for future use.

## Attach the plastic storage pouch to the rear panel

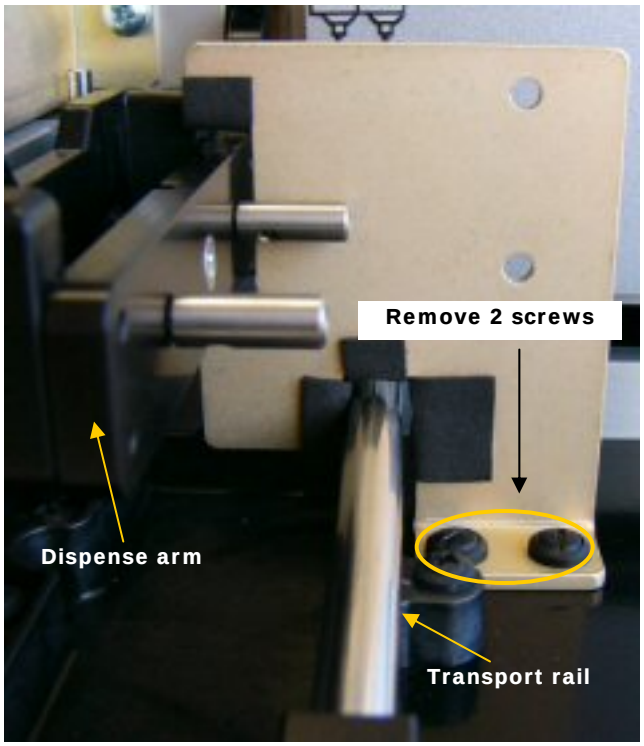
2. Use the Velcro strips to attach the plastic storage pouch to the rear panel of the instrument. Select a position that does not block the vent holes.

## Remove the manifold shipping bracket




1. Use the Phillips screwdriver to remove the 8 screws and washers holding the bracket in place. First remove the screws from the manifold, and then from the instrument.
2. Slide the bracket towards you and remove.
3. Store the bracket, screws and washers with the other shipping material.

## Remove the dispense arm shipping bracket

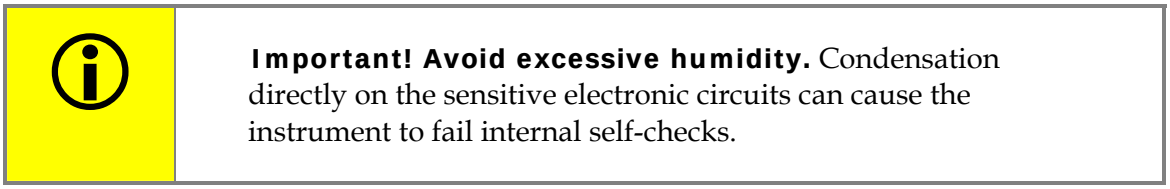



The bracket holds in place both the dispense arm and plate carrier transport rail during shipping.

1. Use the screwdriver to remove the 2 screws holding the bracket in place.
2. Gently lift the dispense arm slightly to release the bracket and remove it.
3. Reinsert the screws into the instrument's base plate to prevent fluid from leaking into it.
4. Store the bracket with the other shipping material.

   The bracket holds in place both the dispense arm and plate carrier transport rail during shipping.

# 3: Set Up the Washer



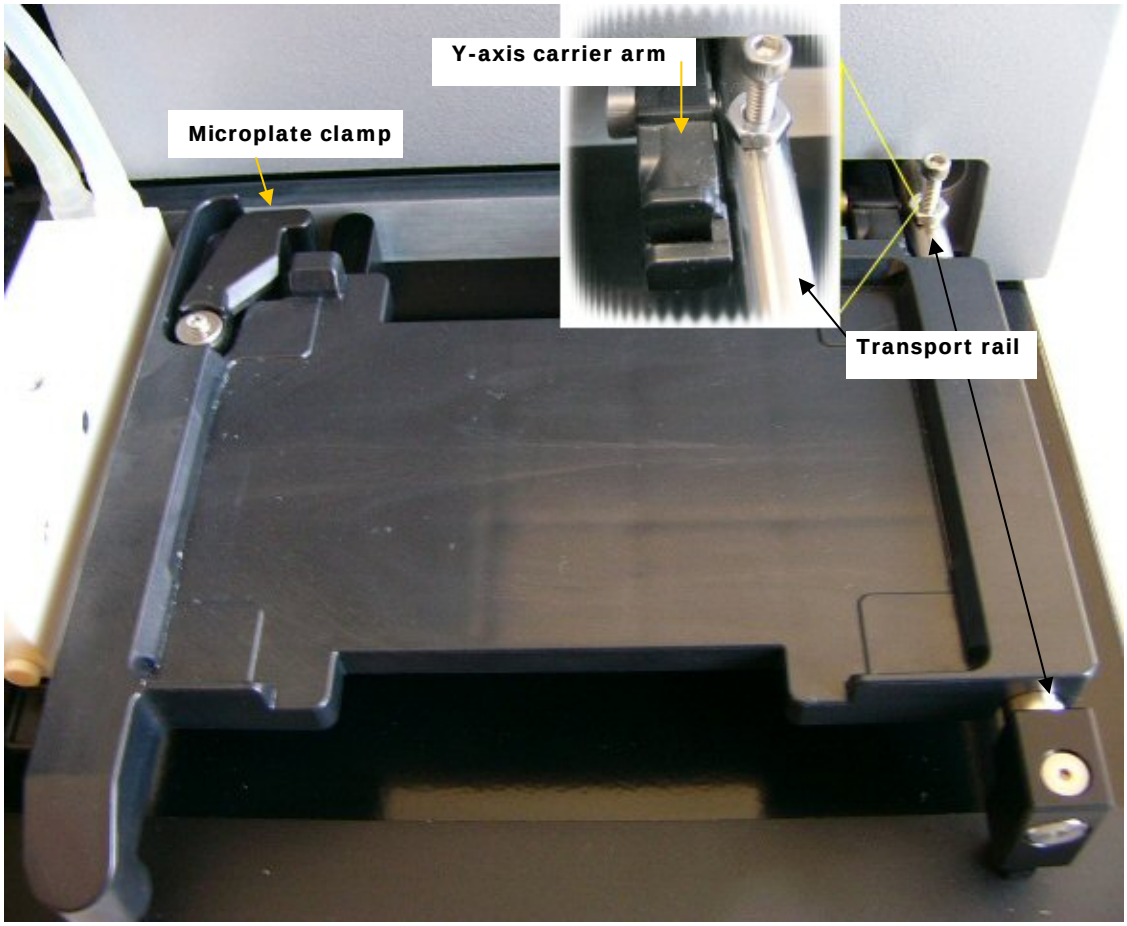
**Important! Avoid excessive humidity.** Condensation directly on the sensitive electronic circuits can cause the instrument to fail internal self-checks.

## Operating Environment

The instrument is sensitive to extreme environmental conditions. For optimal operation, install the EL406 under the following conditions:

- On a level surface.
- In an area where ambient temperatures between 10°C and 40°C can be maintained.
- The instrument should be operated in a non-condensing humid environment having a maximum relative humidity of 80% at temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C.

## Install the Microplate Carrier




The Y-axis carrier arm attached to the Transport rail holds the plate carrier. It moves the plate carrier during processing to allow the dispensers to address high density plates.

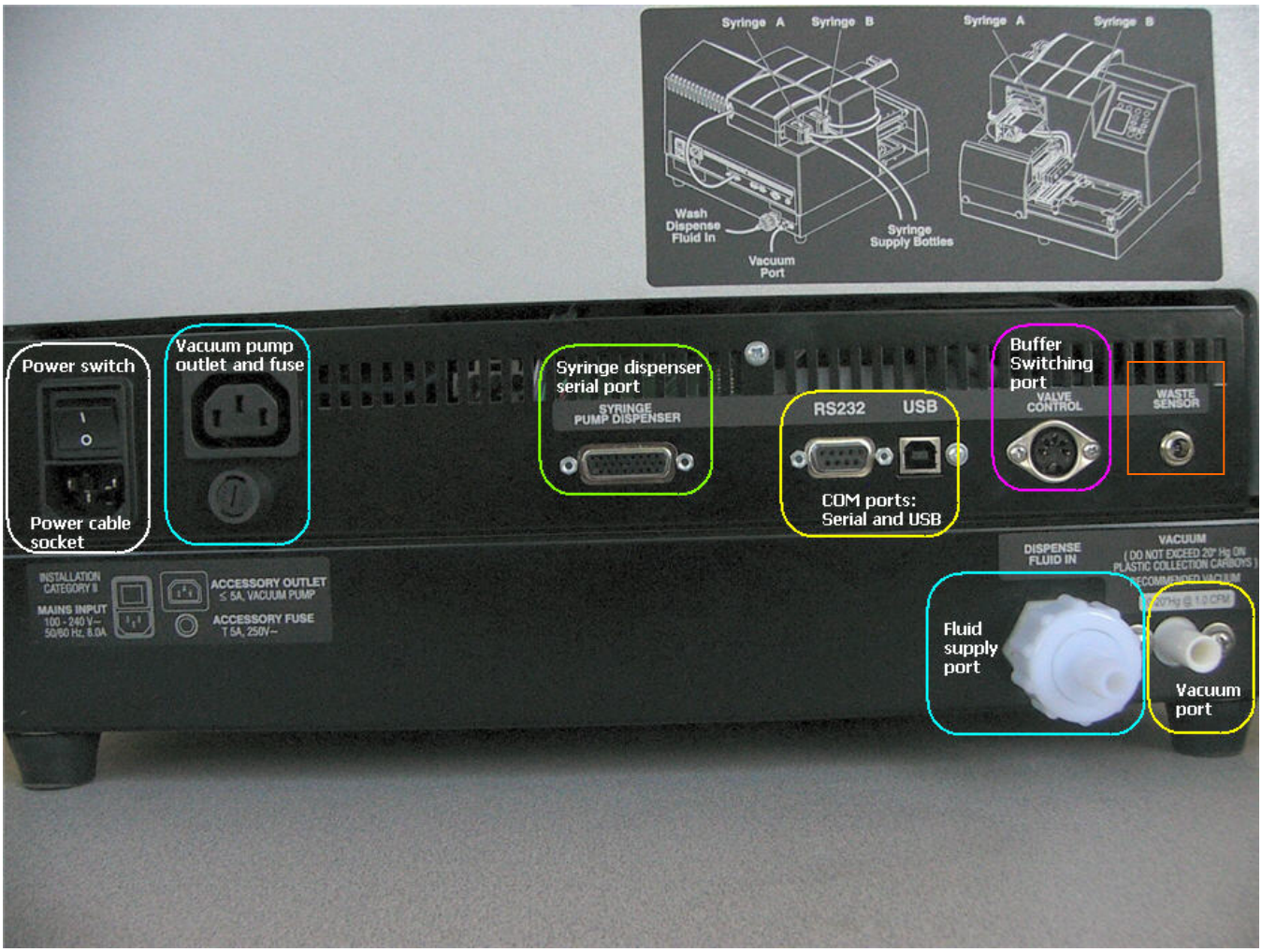
1. Locate the microplate carrier in the accessories box.
2. Slide the plate carrier transport rail all the way to the right, and then back to the left about 1-2 inches (3-4 cm).
3. Look at the underside of the carrier to visualize how it fits in place: its two legs will be positioned on the left and the curved hollow fits over the transport rail on the right. Note the small slit adjacent to the top of the curved hollow. This slit fits into the **Y-axis Carrier Arm**.
4. Aligning the curved hollow with the transport rail, place the carrier on the rail so it fits into the slot on the **Y-axis Carrier Arm**. If necessary, release the spring-loaded microplate clamp in the back left corner of the carrier to level the carrier on the instrument.

❖ If a 401 Instrument Error is displayed at startup, recheck the plate carrier position. Make sure the plate carrier is seated in the **Y-axis Carrier Arm** next to the transport rail.

## Connect the Vacuum Pump, Tubes, and Bottles

For optimal operation of the EL406, all tubing, cables, and fittings for the fluid supply and waste systems must be properly connected. This image illustrates the rear panel of instrument and the locations of the ports and connections for the fluid supply and waste systems.

**Before connecting the tubes and bottles:** Rinse all waste and supply bottles with deionized or distilled water before connecting them to the tubing. Rinsing eliminates particles that may have collected during packing or unpacking.




Rear Panel

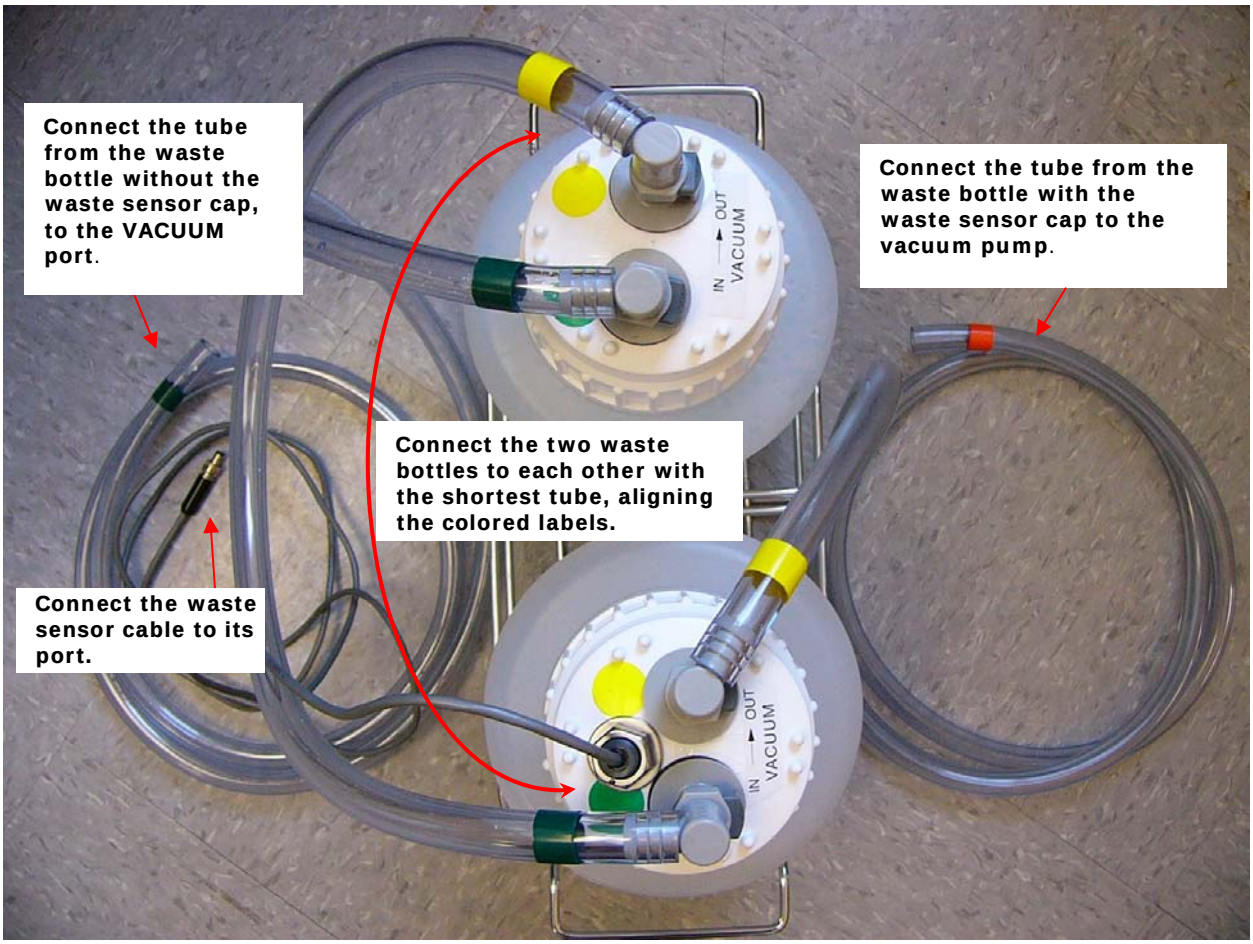
## Waste System

**Caution! Pump Installation.**

**Do not** plug the vacuum pump cable into a wall outlet! Use the adapter provided with the pump to connect it to the **Accessory Outlet** on the back of the instrument. This allows the EL406 to regulate the pump, turning it on and off as specified by the protocol.

When using a standard pump (rather than the high flow pump), set the instrument's **Vacuum Dissipation Delay** to prevent the pump from drawing excess current and blowing the 5-amp fuse. See **Define Instrument Settings** on page 41.

❖ **Note:** The waste tubes have colored bands that match similarly colored dots next to the inlet/outlet ports on the waste bottle caps to ensure the correct connection of the tubing.




Waste System

Three lengths of tubing are shipped with the waste module:

| **Tubing:** | **Connects:** |
|---|---|
| Short tube with yellow and green bands | The two waste bottles to each other |
| Long tube with green bands on both ends | Bottle without sensor to vacuum **port** |
| Long tube with yellow and orange bands | Bottle with waste sensor to the vacuum **pump** |

1. Locate the quick-release caps shipped inside the waste bottles and attach the tubing to them as follows:
2. Connect the waste bottles to each other using the shortest length of tubing, matching the colored bands on the tubing to colored dots on the caps.
3. Attach the waste sensor cable to the **Waste Sensor** port on the back of the washer.
4. Attach the tube from the **waste bottle with the waste sensor** in its cap to the vacuum pump.
5. Attach the tube from the **waste bottle that does NOT have the waste sensor** in its cap to the **Vacuum** port on the back of the instrument.
6. 

**Important!** When installing BioTek's vacuum pump, connect the pump's AC power cable to the vacuum pump **Accessory Outlet** on the back of the instrument (see **Caution** on the previous page). (Use the accessory outlet adapter provided, if applicable.)
7. Place the waste bottles and vacuum pump on the same horizontal plane as the instrument or below it, such as the floor beneath the work surface. This will help optimize performance.
8. Make sure the waste bottle's caps are well sealed.

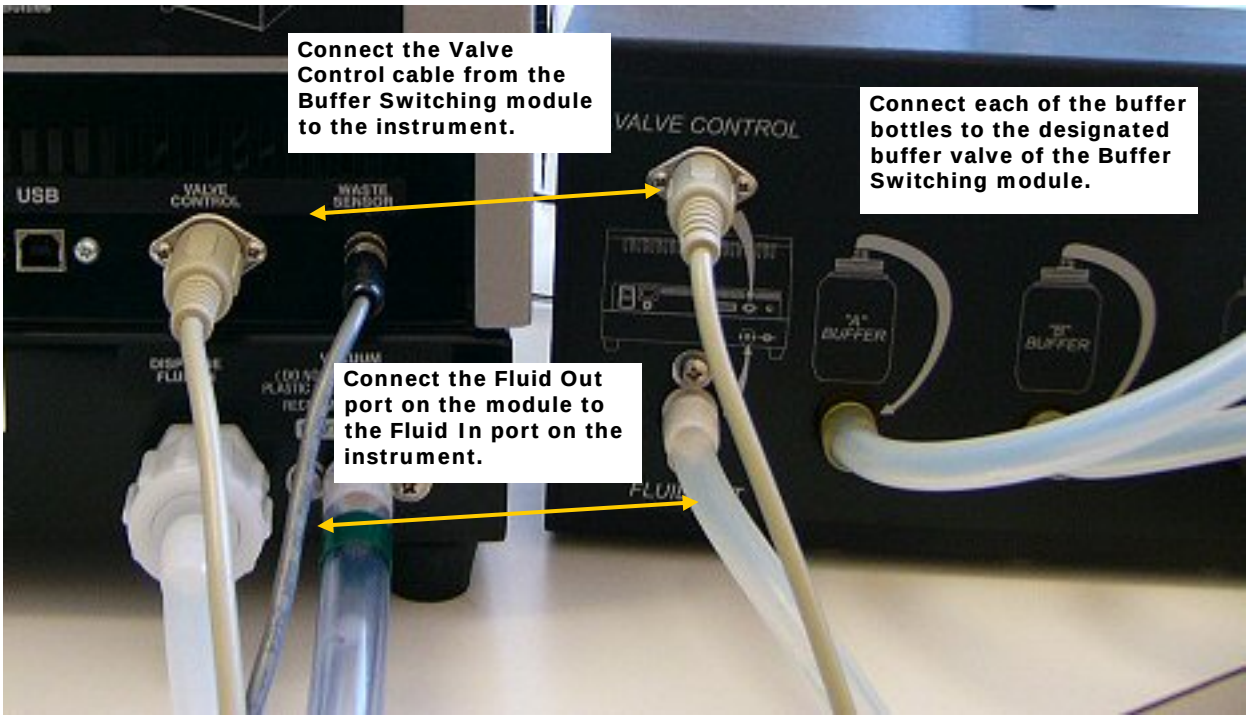
## Install the Optional Vacuum Line Filter (PN 48294)

The optional vacuum line filter can be installed halfway between the last waste bottle (overflow bottle) and the vacuum pump.

To do this, cut the tubing and insert the filter, noting the direction of flow. The flow arrow on the filter should point **toward the vacuum pump**.

In the event of a fluid overflow, the filter should prevent the destruction of the vacuum pump's internal components. If an overflow does occur, check the filter for trapped fluid. If fluid is found in the filter, remove the filter and drain using the small white nut on top of the filter. Tighten the white nut and reinstall the filter.

### *Fluid Supply System with the Buffer Switching Module*




Buffering Switching System

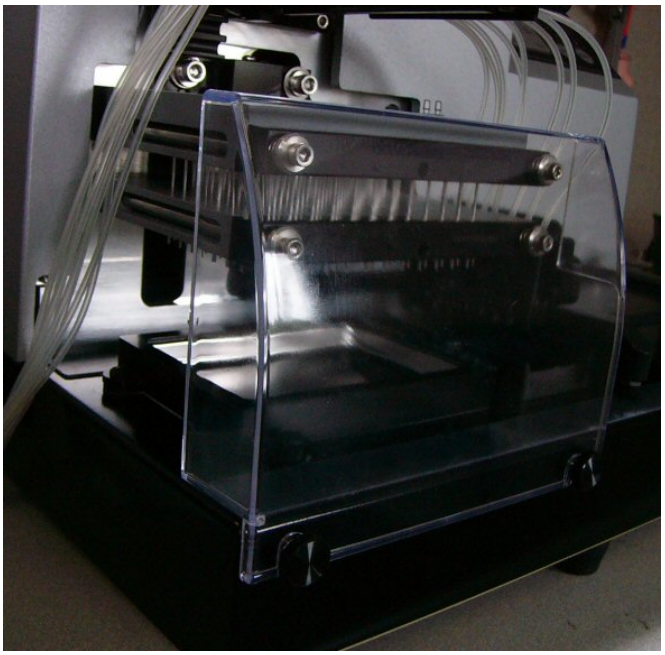
1. Connect the valve cable from the **Valve Control** port on the module to the **Valve Control** port on the back of the instrument.
2. Place the four supply bottles and valve module on the same surface as the instrument to optimize performance.
3. Connect the tubing from one of the supply bottles to "A" Buffer in the valve module.
4. Repeat step 3 with the other three supply bottles for "B," "C," and "D" Buffers.
5. Connect the 6-foot (1.83 Meter) tubing from the valve box **Fluid Out** port to the **Dispense Fluid In** port on the instrument's rear panel. This tubing can be cut to the optimal length required for the installation.

❖ **Note**: Most instruments are configured at the factory to use the Buffer Switching Module to supply fluid. If you choose not to use the module and instead connect a supply bottle directly to the instrument, the volume dispensed to the plate will be slightly larger than specified, approximately 3-7% larger.

### *Final Check*

- Verify that the tubing was not crimped during installation.
- Ensure that there are no loose fittings or cable connections.

## Attach the Mist Shield



Mist Shield

1. Loosen the two thumbscrews in the front base of the instrument, directly in front of the washer manifold and priming trough.
2. Position the mist shield so the gaps align with the thumbscrews. One side of the mist shield rests on the instrument base.
3. Finger-tighten the two thumbscrews to hold the shield in place.

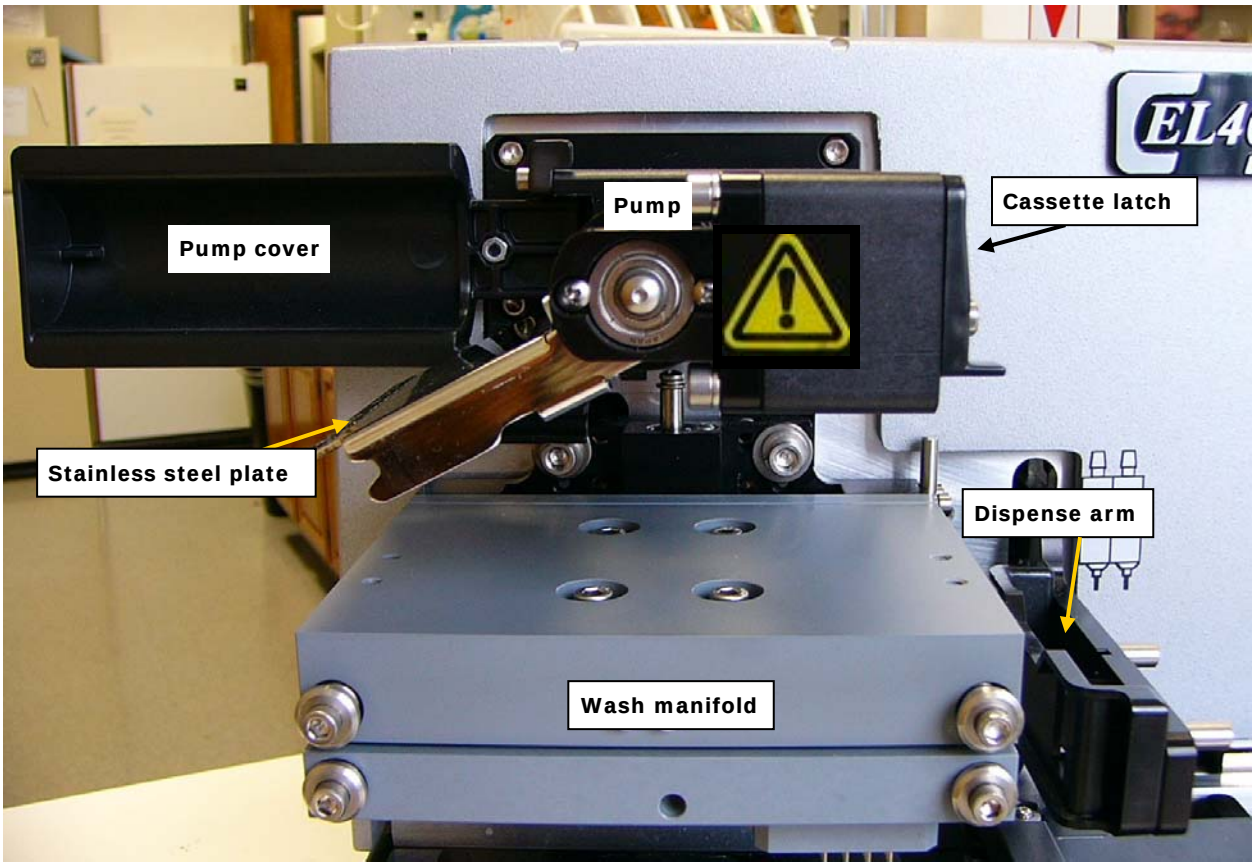
# 4: Set Up the Peri-pump Dispenser

Install these items to use the Peri-pump dispenser:

- Tubing cassette
- Fluid supply vessel
- (Optional) Prime trough insert

## Install the Tubing Cassette

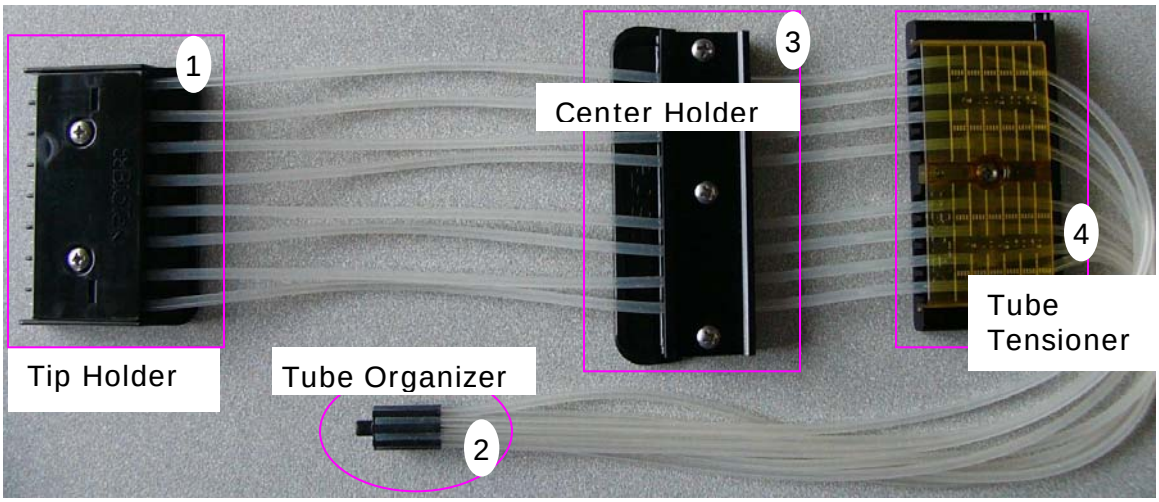
Take a moment to identify the EL406's parts that are named in the instructions for installing the tubing cassette.




To prepare the dispenser for cassette installation, open the **pump cover**, release the **cassette latch** and lift the **stainless steel** plate up and over, to hang freely.

❖ The pump cover must be closed to operate the dispenser.

## Tubing Cassette Diagram

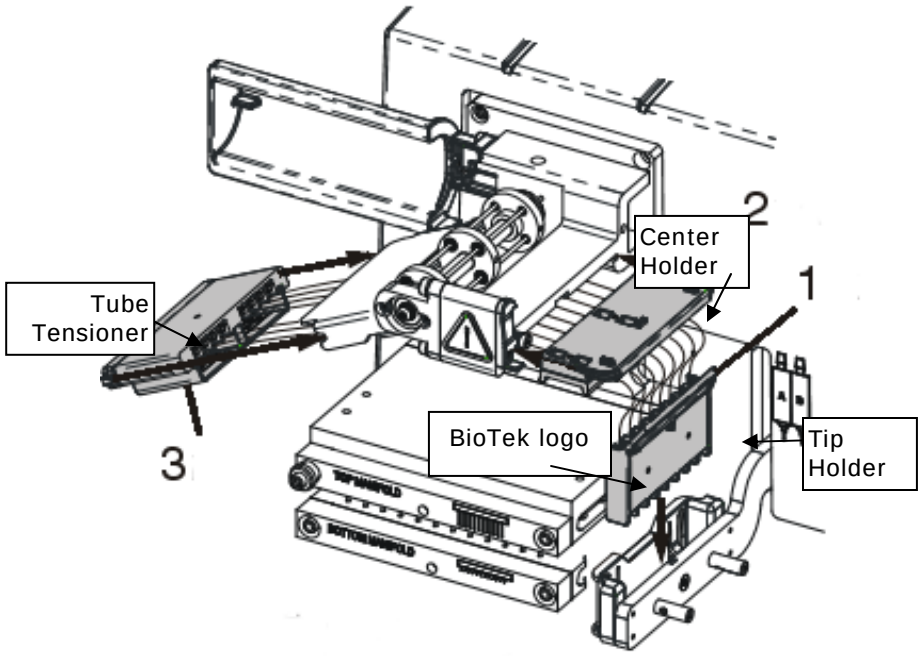



Cassette Diagram

1. **Tip Holder**: The cassette's easiest part to identify, the tip holder fits into the dispense arm to the right of the pump for positioning above the plate.
2. **Tube Organizer**: At the opposite end of the cassette from the tip holder, the tube organizer holds the 8 tubes together for inserting into the fluid vessel.
3. **Center Holder**: The center holder is labeled to identify the size of the cassette tubing. It also has a serial number for tracking purposes. It fits in between the tip holder and the tube tensioner and fixes the tubes in place. It slides into grooves on the underside of the pump.
4. **Tube Tensioner**: The transparent 5-mm scale on its front surface identifies the tube tensioner. It has 8 internal screws for stretching the tubing, one for each tube. The tube tensioner's scale is useful when calibrating the cassette.

### Prerequisites:

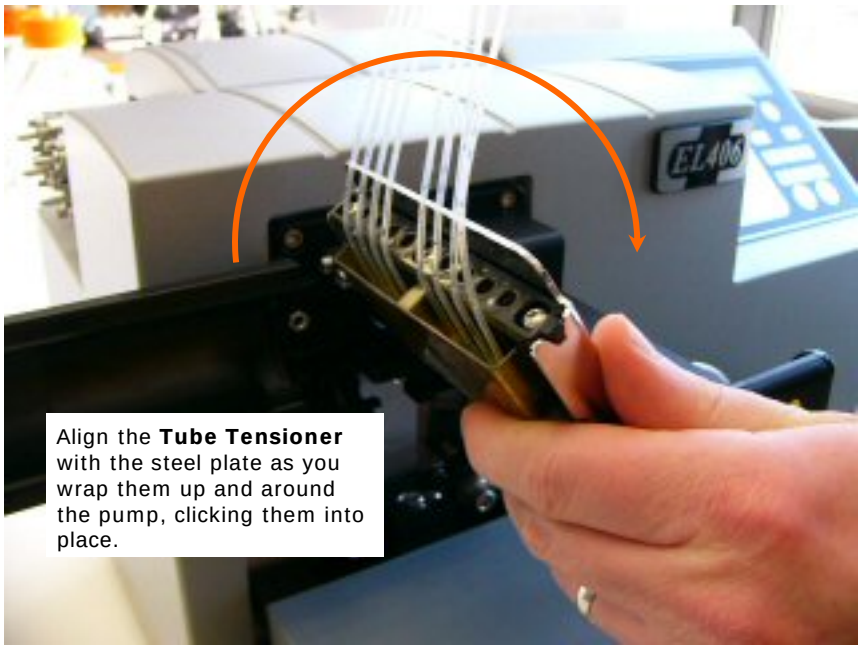
- Review the **Tubing Cassette Diagram** above to learn the names of the components.
- Move the **Pump Cover** away from the pump to its OFF position.
- Release the pump's stainless steel plate:
- Release the spring-loaded latch on the right–side of the caution symbol (  ) on the pump and lift the stainless steel plate up and out.



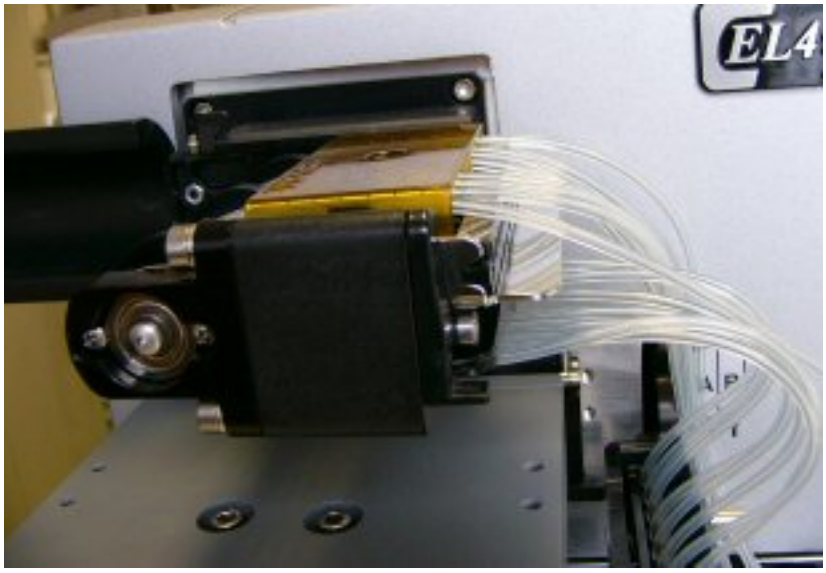

Installing the Peri-pump Dispense Cassette

1 Slide the **Tip Holder** into the dispense arm. The Tip Holder's front plate with the BioTek logo faces the pump. Make sure the tip holder is level and snapped into place.


2 Extend the rest of the cassette under the Peri-pump and above the washer manifold, so you can slide the **Center Holder** into its slot on the underside of the pump. A tab on the back plate of the center holder fits into a notch on the pump. The label on the center holder faces away from the pump.

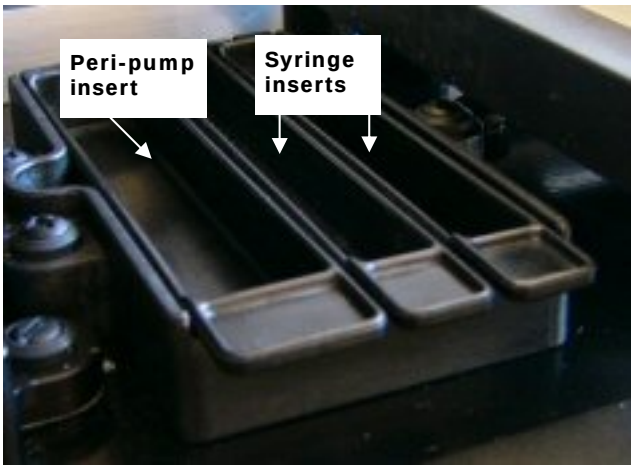



5. Align the **Tube Tensioner** with the stainless steel plate, making sure the knobs on top of the tensioner fit correctly into the grooves. Hold the cassette and steel plate together as you wrap them around and up against the pump and click the steel plate into place.



6. Return the **Pump Cover** to its **RUN** position covering the pump.
7. Lift the **Tube Organizer** over the pump cover. Place it in the fluid vessel, when you're ready.

## Prime Trough Insert




Priming Trough Inserts

The EL406 ships with reservoirs that fit into the dispensers' priming trough to capture expensive reagent after priming, rather than discarding it. Three **Prime Trough Inserts** are provided:

- PN 7182043 for the Peri-pump dispenser holds approximately 12 mL
- PN 7182044 (2) for the Syringe dispensers holds approximately 6.5 mL

❖ Without the prime trough insert, the priming trough empties into the regular waste bottle described on page 24.

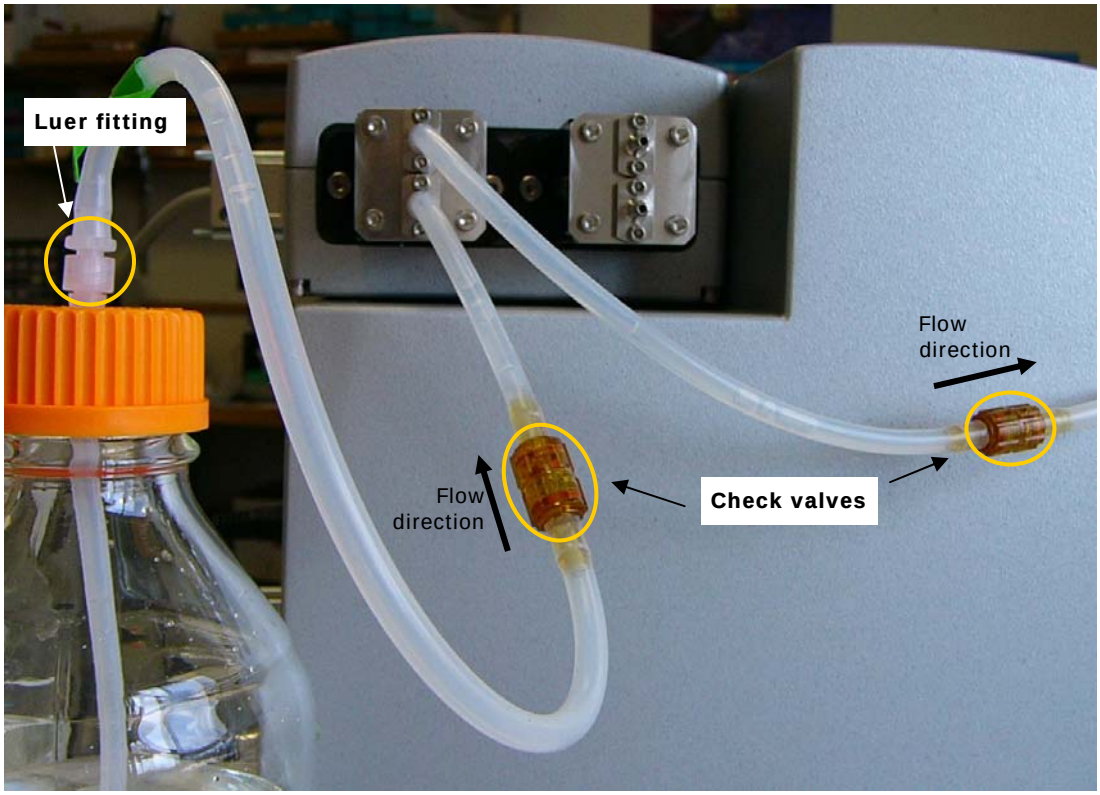
# 5: Install the Syringe Dispenser

- The dual Syringe Dispenser is an optional component. Skip this section of the installation process if it is not applicable.
- 1536-well EL406 models ship with both the 16-tube dispense manifolds, which dispense to 96- and 384-well plates, and the 32-tube dispense manifolds for processing 1536-well plates.

The Syringe dispenser is an optional component and ships separately.

### To install the unit:

1. Inspect and unpack the shipping container.
2. Place the unit on top of the EL406 in the hollow designed to hold it (top left, with special indentations for the unit's feet). Alternatively, place the unit on the same surface as the instrument.
3. Plug the 26-pin high-density cable into the back panel of the EL406 in the port labeled **Syringe Pump Dispenser**. Plug the other end into the dispenser unit.
4. Connect the tubing:




### *Check Valves, Tubes, and Bottles*

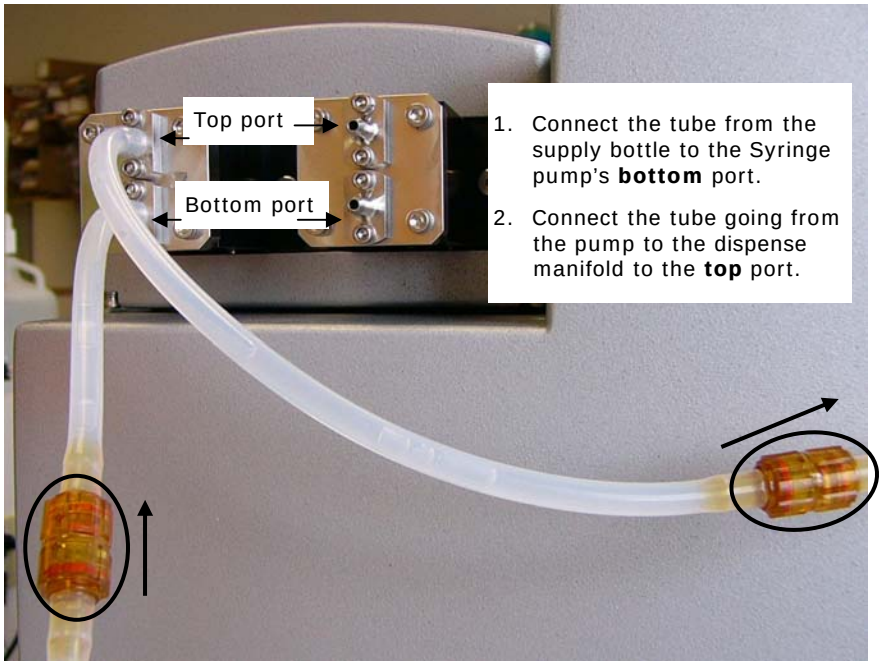
For each Syringe dispenser, two sets of tubing with check valves and a two liter supply bottle are provided. The 32-tube dispense manifolds also ship with an

optional inline filter that BioTek recommends installing when using the 32-tube SB – small bore – models. Learn more about check valves on page 35.

| | |
|---|---|
| ⚠ | The supply bottles have Luer fittings. **Finger-tighten only!** |

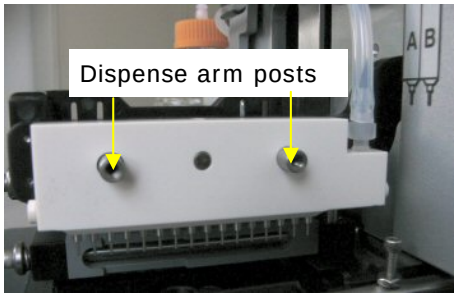
- Rinse all bottles with deionized or distilled water before using to eliminate particles that may have entered during packing or unpacking.
- Place the supply bottles on the same horizontal plane as the instrument. This ensures optimum pump performance.
- Verify that the tubing was not crimped during installation.

5. Locate the set of tubing with the Luer fitting on one end. (After installing the inline filter for 32-tube dispensers, if applicable), gently screw the Luer fitting into the top of the supply bottle. Finger-tighten only.
6. Attach the other end of the tubing from the supply bottle to the **bottom port** of one of the Syringe pumps.



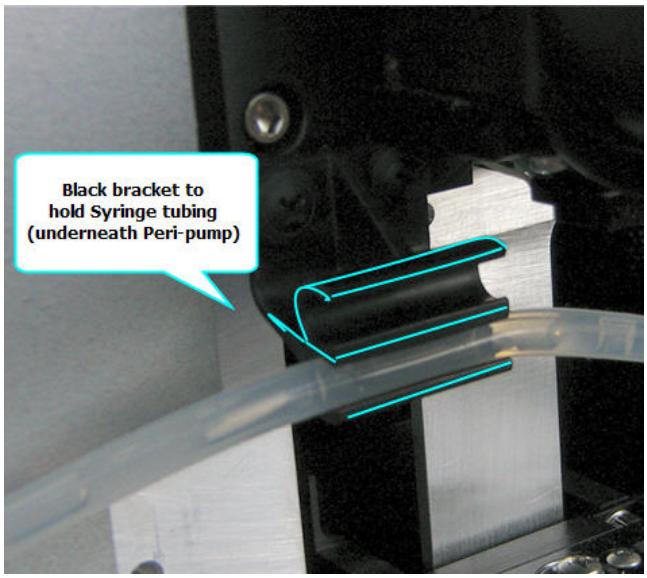

Make sure the flow-direction arrows on the check valves point in the right direction.

7. With the check valve's flow-direction arrows pointing correctly, connect the other tube to the top port of the Syringe pump.
8. Connect the tube's other end to the dispenser manifold.




9. Slide the manifold onto the two posts on the dispense arm with the tubing end closest to the instrument. Install Syringe A's manifold first. Syringe B's manifold must be installed after Syringe A's. See the instructions for removing the magnets for certain assays on page 35.

❖ The images of syringes labeled A and B on the instrument indicate the placement of each dispenser's manifold: A slides on first, then B.




10. Use the black bracket attached to the instrument just below the Peri-pump to keep the tubing out-of-the-way. Make sure the manifold has enough slack in the tubing to move freely, and then snap the tubing into the bracket.

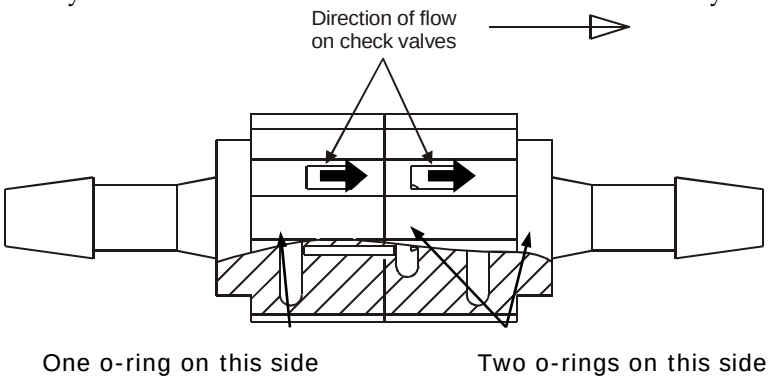
11. Repeat the check valve and tubing installation for Syringe B.

**Important!** If the dual **Syringe Dispenser** unit was purchased separately, e.g., at a later date, from the instrument, you must tell the EL406 that it is installed, i.e. use the Instrument Utilities to update the instrument's internal basecode. Find instructions on page 42.

### Check Valves

Note the flow direction arrows on the check valves. Some are harder to see than others.

- PN 68083 – Autoclavable valves for use with non-organic substances. The direction arrows are difficult to see.
- PN 68073 – Check valves recommended for use with organic substances. They cannot be autoclaved. Direction arrows are easy to see.




Autoclavable check valve, detail

It is difficult to see the flow direction arrows on the autoclavable check valves. The valves are made of a translucent plastic, in which the flow direction arrows are engraved.

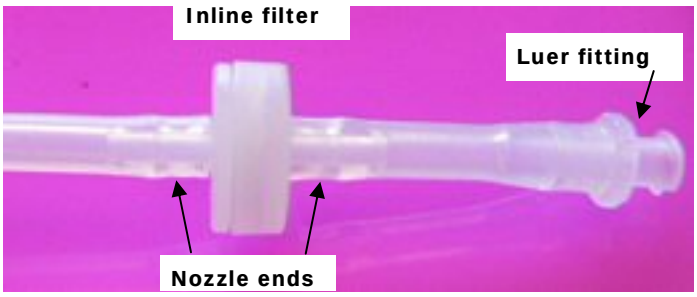
❖ Note: If the check valves are replaced, it may be necessary to recalibrate the syringe backlash to achieve optimum performance. See "Recalibrate the Backlash" in ***Chapter 4*** for more information.

## Install Inline Filter for 32-Tube Dispensers

BioTek ships two 90-micron inline filters with the 32-tube dispense manifolds to reduce the chances of clogging the dispense tubes. It is especially important for the SB – small bore models.

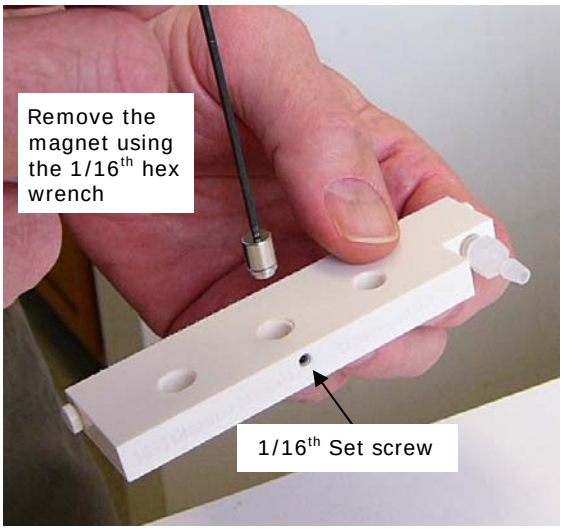
To install the filters:

1. Locate and layout the length of tubing that goes between the supply bottle and the pump; it has the Luer fitting on one end.
2. Cut the tubing approximately one to two inches above the Luer fitting.
3. Slide the filter's nozzle ends into the two ends of the tubing, reconnecting it.

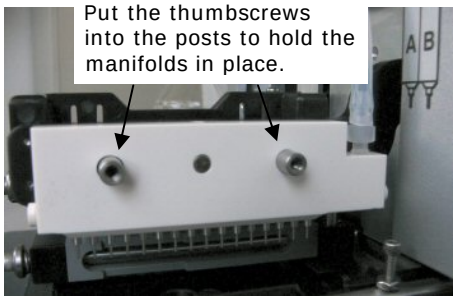


### Special Procedure for Magnetic Bead Assays

Before using the dual **Syringe Dispenser** to dispense magnetic beads, you must remove the magnets that normally hold the dispense manifolds on the instrument. Two thumbscrews are shipped with the instrument to replace the magnets.




1. Use the 1/16th hex wrench (PN 48713) to loosen the set screw on top of the manifold.
2. Take advantage of the magnet's attraction to the hex wrench to remove it from the manifold.
3. Locate the thumbscrews shipped with the instrument's accessories. After sliding both manifolds onto the dispense arm, put the screws into the post to hold the manifolds in place.




❖ Store the magnets in the plastic pouch for potential future installation.

# 6: Install the LHC Software/Connect to Computer (Optional)

For a more graphically rich experience designing protocols and monitoring progress, the EL406 can be controlled with software for your personal computer (PC):

- **Liquid Handling Control™ (LHC) Software**
- **EL406™ Interface Software (EL406 IS)**
- **BioStack™ PC Control Software** (if applicable)

Please refer to the ***LHC Installation Guide*** for complete installation and setup instructions. Likewise, install the **EL406 Interface Software** and **BioStack PC Control Software** (if applicable) according to instructions provided with the CD.

## Connect the USB or Serial Cable to the Host Computer

Two cables are shipped with the EL406:

**If using the serial cable:** Plug one end into the **RS232** serial port on the rear of the instrument and the other end into an available port on the computer.

**If using the USB cable:** Plug one end into the **USB** port on the rear of the instrument and the other end into an available port on the computer.

- If the computer is connected to the internet, turn on the instrument. Windows® will automatically locate and install the necessary USB drivers (follow the instructions provided on the screen).
- If the computer is NOT connected to the internet, you can install the drivers using the supplied "Virtual USB Com Port" driver software CD.
- Virtual Com Port (VCP) drivers for **all** Windows operating systems are available at http://www.ftdichip.com/Drivers/VCP.htm

❖ The EL406's keypad must be displaying its "Main Menu" for the LHC to communicate with it.

# 7: Connect to Power

| | |
|---|---|
|  | **Warning! Power Rating.** The EL406™ must be connected to a power receptacle that provides voltage and current within the specified rating for the system. Use of an incompatible power receptacle may produce electrical shock and fire hazards. |
|  | **Warning! Electrical Grounding.** Never use a two-prong plug adapter to connect primary power to the EL406. Use of a two-prong adapter disconnects the utility ground, creating a severe shock hazard. Always connect the system power cord directly to a three-prong receptacle with a functional ground. |

The EL406 supports voltage in the range of 100-240 V~ at 50-60 Hz.

1. Insert the power cable into the power cable socket in the rear panel of the EL406.
2. Insert the three-prong plug into an appropriate receptacle.

# 8: Define Instrument Settings

## Standard Vacuum Pump Users

> ❖ **Do not** perform this step when using the **High Flow** vacuum pumps (PN 7100563, 7100564). High flow pumps are recommended when aspirating 384- and 1536-well plates with wash buffers not containing surfactant (e.g., pure DI water).

Perform this step **ONLY** if you are using the "standard" vacuum pump (BioTek PN 7100561, 7100562): increase the **Vacuum Dissipate Delay** to match your waste container: 1 second per liter. For example, if you have a 10 L waste bottle, set the delay to 10 seconds.

| Option | Description |
|---|---|
| **Using the keypad** | 1. Press **Setup Menu**.<br>2. → Select the arrow (for more options) twice.<br>3. Select **VACDIS**.<br>4. Use the number pad to set the **Vacuum Dissipate Delay** to match your waste container: 1 second per liter.<br>5. Press **Main Menu** upon completion. |
| **Using the LHC**<br>  | 1. Select **Tools > Instrument Utilities**.<br>2. Under "General Settings," increase the **Vacuum Dissipate Delay** to match your waste container: 1 second per liter.<br>3. Click **Send** to download this new setting to the instrument.<br>4. Click **Exit** to return to the main screen. |


## LHC Users Only



When using the LHC to control the EL406, an important first step is defining your instrument's settings. After installing the LHC and EL406 IS software, you can use the desktop icon or from the Windows Start button to launch the LHC:


**> All Programs> BioTek> Liquid Handling Control**

1. Click the **Name** link on the main page and, if required, select the EL406.
2. Specify the COM **Port** used to connect the EL406 to the computer and click **Test Communication**.
   - **Pass**: proceed to the next step.
   - **Fail**: check the Com Port setting. See "About Com Ports" in the LHC Help.
3. In the Instrument Settings dialog that opens, under Get settings from: click the **instrument** link, and click **OK**.

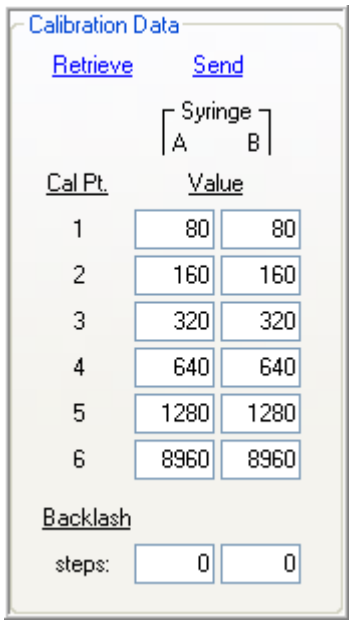
## Update the EL406 to use the Syringe Dispenser

> ❖ **Important Prerequisite**: The EL406 Interface Software (IS) is required to update the instrument's configuration data. You must be controlling the EL406 with the LHC software or obtain a copy of the EL406 IS Utility from BioTek's customer resource center: https://customer.biotek.com.

If the dual Syringe Dispenser unit was purchased separately from the instrument, you must update the EL406's internal basecode. When the Syringe dispenser is ordered with the instrument, BioTek updates the basecode at the factory.

> ❖ **1536-well models**: All Syringe dispensers are calibrated for use with their 16-tube dispense manifolds. When dispensing with the 32-tube dispensers, if dispense accuracy needs improvement, make a small adjustment to the Backlash to achieve specified performance.

1. Turn on the instrument, launch the LHC software or the EL406 IS Utility and make sure it is communicating with the EL406 (define the correct COM port).
2. Select **Tools>Instrument Utilities>Syringe Dispenser**.
3. Select the **Installed** button under Syringe Pump Assembly and click **Send**.
4. Review or enter the Calibration Data:




**Review the data**:

BioTek enters the Calibration Data and tests the syringe before shipping when it is purchased with the EL406. In this case, you may want to verify the correct values have been entered.

Compare the data onscreen to the data sheet on the bottom of the modular unit.

**Enter the data**:

If you have purchased the syringe module separately, you must enter the Configuration Data for the unit. Find the data sheet on the bottom of the syringe dispenser module and enter the values in the Cal Pt. table.

**After entering data points, click Send to download them to the instrument.**

> **Tip:** Small adjustments to the **Backlash** may be needed to improve the dispense accuracy of 32-tube dispense manifolds: See **Chapter 4, Maintenance** for "Recalibrate the Backlash" instructions.

# 9: Verify Performance

Before using the ELx406 for the first time, verify that it is operating properly.

- When using the LHC, make sure the EL406 is connected to the PC and both are powered up.
- When running standalone, turn on the EL406.

### Using the keypad:

1. Press the Setup Menu button, and select → for more options.
2. Select TESTS and SLFCHK.

### Using the LHC:

1. Click the Name link on the main page and, if required, select the EL406.
2. Define the COM Port used to connect the EL406 to the computer and Test Communication.
   - **Pass**: proceed to the next step.
   - **Fail**: check the Com Port setting. See "About Com Ports" in the LHC Help.
3. In the Instrument Settings dialog that opens, under Get settings from: click the instrument link, and click OK.
4. Select Tools>Instrument Utilities
5. On the General Settings tab, click the Perform Self-Check link.0.

### Test results:

- **Pass**: a passing message is displayed.
- **Fail**: an error message is displayed. If this happens, note the error code and refer to ***Appendix A, Error Codes*** in the operator's manual to determine its cause. If the problem is something you can fix, turn off the instrument, fix the problem, and then turn the washer back on. Otherwise, contact BioTek's Technical Assistance Center.

***Chapter 5, Qualification*** in the operator's manual provides recommended Installation and Operational Qualification procedures to be performed after the instrument is installed and *before* the instrument is used in a laboratory environment.

The successful completion of the Installation Qualification confirms that the EL406 and its components have been supplied as ordered, and assembled and configured properly for your lab environment.

The successful completion of the Operational Qualification confirms that the EL406 is operating according to specification.

❖ **Note:** An instrument qualification package (PN 7180527) for the EL406 is available for purchase from BioTek. The package contains thorough procedures for performing Installation Qualification, Operational Qualification and Performance Qualification (IQ-OQ-PQ) and preventive maintenance (PM). Extensive Checklists and Logbooks are included for recording results. Contact your local dealer for more information.

**Important!** Before operating this EL406, review the ***Optimize Performance*** guidelines in ***Chapter 3, Operation***. These guidelines include necessary steps to perform before running a protocol, information on performing periodic maintenance, and points to consider when creating or editing washer programs.

# Define Startup Preferences (LHC users only)

You can save enormous time creating protocols by following these steps to define a **New Protocol** template and use it at startup.

## Create a protocol template

1. Click the **New** button or select **File>New**.
2. Click **Name**. Select the instrument, if you have more than one, and define its **Port** and **Settings**.
3. Optionally, select the Plate Type, fill in the text fields, and add any steps that you want all new protocols to include.
4. Click **Save** and assign a unique name, e.g. Template.LHC.
5. Select **Tools>Preferences>New Protocol**.
6. Select the button for Protocol selected below to use as a template.
7. Click **selected** and select the protocol you created as a template.

## Define startup behavior:

8. After completing the steps above, select the Startup Options tab.
9. Select the button for **New Protocol**.
10. Click **OK** to save your new preferences.

# Repacking the EL406

- **Failure to comply with the following instructions will void the instrument's warranty.**
- **Follow the decontamination procedure in *Chapter 4, Preventive Maintenance*, before returning the instrument.**

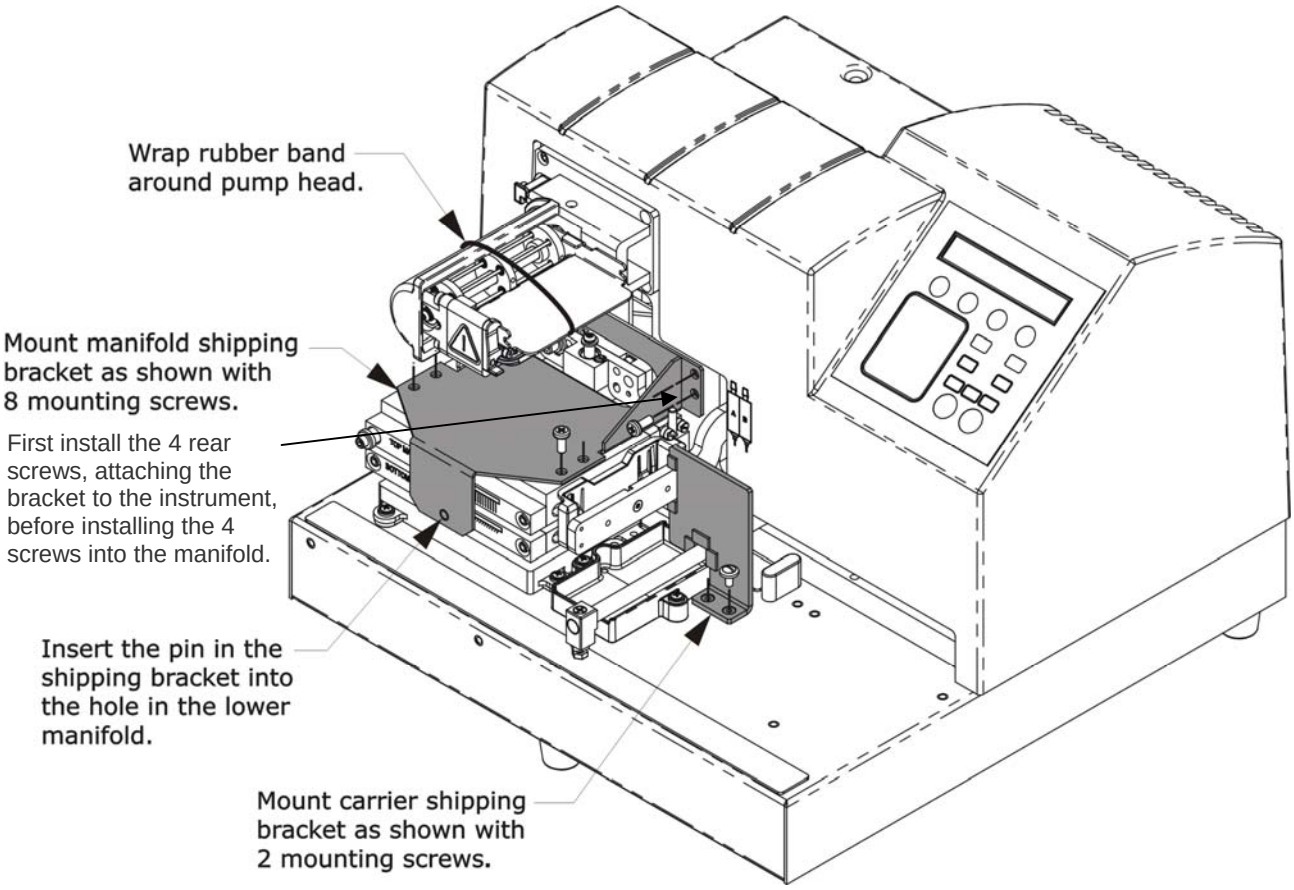
## Prepare the instrument for shipping bracket installation:

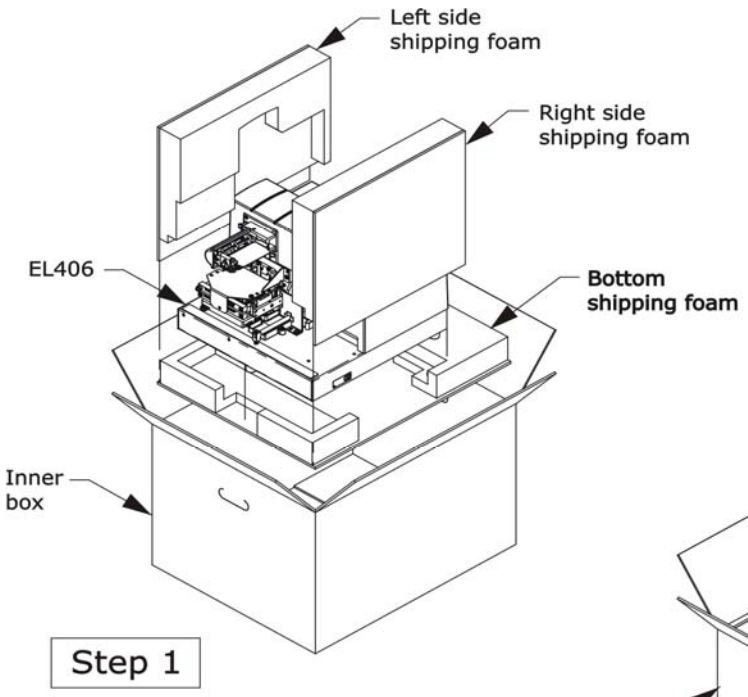
| Using the | Description |
|---|---|
| **Keypad** | 1. Press **Setup Menu**.  <br>2. Select →<br>3. Select **WASH**.<br>4. Select **PARK**. |
| **LHC** | 1. Select **Tools>Instrument Utilities>Washer.**<br>2. Click the **shipping bracket** link under Service Functions. |

## Continue to prepare the instrument for shipping:

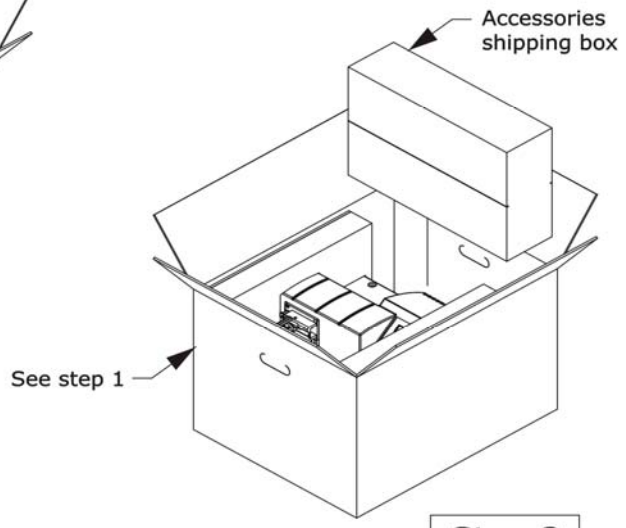
1. Remove the Peri-pump's cassette and the Syringe dispensers' manifolds, if applicable.
2. Remove the **plate carrier** and put it in the accessories box.
3. Slide the transport rail into position next to the dispense arm. It will be secured in place by one of the shipping brackets.

## Install the shipping brackets:



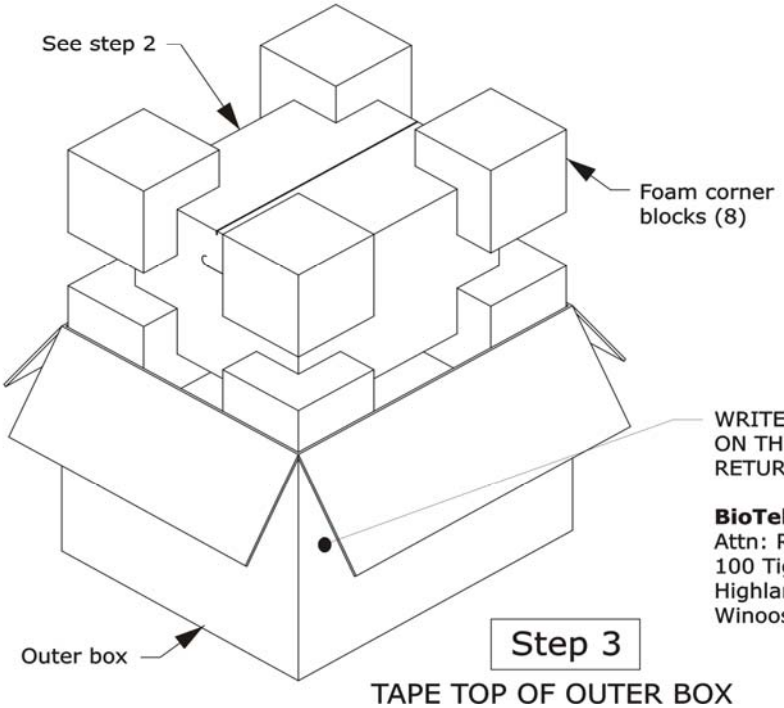



Step 1




Step 2

TAPE TOP OF INNER BOX




WRITE "RMA" IN BIG CLEAR LETTERS ON THE OUTSIDE OF THE BOX AND RETURN TO:

**BioTek Instruments, Inc.**
Attn: RMA# xxxxx
100 Tigan Street
Highland Park
Winooski, VT 05404-0998

Step 3

TAPE TOP OF OUTER BOX

❖ The instrument's packaging design is subject to change over time. If the instructions in this section do not appear to apply to the packaging materials you are using, please contact BioTek's Technical Assistance Center for guidance.

Chapter 3

# Operation

This chapter covers basic operation of the EL406 using the LHC™ software and the instrument's keypad, and provides guidelines to ensure the instrument's top performance.

# Basic Operation

❖ Review the EL406 Installation Guide or Chapter 2, Installation, for instructions on how to set up the fluid supply, vacuum pump, and waste receptacles. This section describes how to use the keypad or the LHC to run the instrument.

## Two ways to control the EL406

You can control the EL406 using its built-in keypad or with BioTek's **Liquid Handling Control™ (LHC)** software, which works with the **EL406™ Interface Software (IS)**, to design protocols and control the instrument.

This section includes a quick introduction of the instrument's keypad, a complete description of keypad functionality is provided later in the chapter.

To use the LHC to control the EL406, it must be attached to and communicating with your personal computer (PC), and its main menu must be displayed. Basic protocols can be created or modified using the LHC, and then downloaded to the instrument for stand-alone operation.

| Using the | Learn about the options: |
|---|---|
| **Keypad** | ➢ Introducing the Keypad on the next page.<br>➢ Controlling the EL406 using the Keypad on page 66. |
| **LHC** | ➢ Introducing the LHC – EL406 Workspace on page 72.<br>➢ Use the LHC Help system: select the **Help** menu or click a **Help** button in a window. |

❖ When using the LHC, make sure the required software has been installed and other installation tasks have been completed.

## Turn On/Off the EL406

To turn the instrument on or off, press the On/Off switch on the rear panel:

**|** = **On** ○ = **Off**

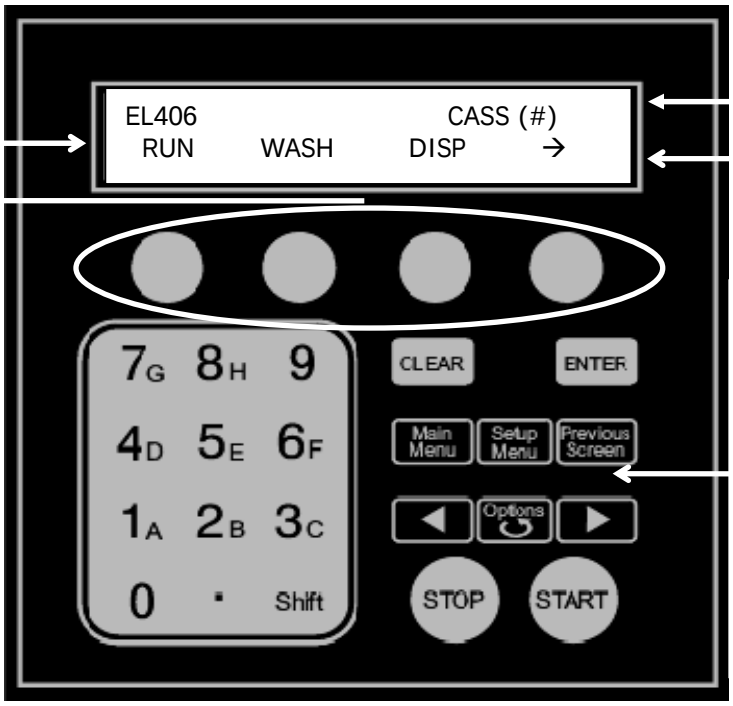
The EL406 performs a self-test.

- If the test passes, the Main Menu will appear and the instrument is ready for use.
- If the self-test fails, the EL406 will "chirp" and display an error code. Make sure nothing is obstructing the movement of the plate carrier and washer manifold and restart the instrument. If the error persists, look up the code in ***Appendix A: Error Codes***.

## Introducing the Keypad

> ❖ This is a quick introduction to the keypad. Find complete instructions for using keypad beginning on page 66.

The EL406 features a keypad and 2-line x 24-character LCD display. The main menu is represented here:




Starting at the top of the keypad, note the main menu and the **Soft-keys**. Use the Soft-keys to make selections. To return to the main menu, press the **Main Menu** key.

- **RUN** – to run a defined protocol. Use the **Options** key to select a protocol or enter its number. Review the list of pre-defined protocols on page 97.
- **WASH** – to run a quick wash. Put a plate on the carrier, set the desired parameters and click **Start** to run a wash routine. Except when the 1536-well hardware is installed and quick wash is not supported.
- **DISP** – to run a Quick Dispense. Dispense options using the Peri-pump are offered first. Press **Pump** to dispense with one of the Syringe dispensers, if applicable.
  - **Peri-pump Dispenser Quick Dispense options**:
    - Press and hold the **Prime** and **Purge** keys to execute these actions for as long as you hold the key.
    - Set the **dispense Volume** using the arrow and number keys, put a plate on the carrier, and press **Start**.
    - First, make sure the **CASS (#)** matches the installed cassette type. If not, change the setting as described on page 50.

- **Syringe Dispenser Quick Dispense options**: When the dual Syringe dispensers are installed press **Pump** at the quick dispense menu to toggle through the list of dispensers, Syringe A, Syringe B, and the Peri-pump.
  - Press the **Prime** key to prime the tubing with **5000 µL** of fluid.
  - Set the **dispense Volume** using the arrow and number keys, put a plate on the carrier, and press **Start**.

- **PLATE –** The default plate type for Quick Wash and Dispense is 96-well. Select **PLATE** to change it, then scroll to the correct type. Learn more beginning on page 54.
- Setup Menu **Setup Menu**: press this key to access the instrument's general settings and the settings for each of the devices and the BioStack; to run system tests or the Adjust Utility. Turn to page 79 for details.
- 

The **Options** key (and sometimes the arrow keys) scroll through the available options or settings for the current focus. **Shift+Options** reverses the scrolling direction.
- → : Select the arrow key in a menu to reveal more options.
- **Define**: leads to the protocol creation and editing mode, which is described on page 66.

## Stopping an Operation

| Using the | Description |
|---|---|
| **Keypad** |   Press the **STOP** key. |
| **LHC** |   Click the **STOP** button. |

Both actions require confirmation. You will be prompted to **Stop** or **Resume**. Press Stop again to cancel the currently running program. Otherwise, choose Resume.

**Important!** Settings defined onboard the instrument must match its physically installed components. Be sure the washer's manifold, 96-Tube, 128-Tube, or 192-Tube, and the Peri-pump's cassette (CASS), 1 µL, 5 µL, or 10 µL, are properly defined **before** operation. Operating the washer with the wrong setting may damage the manifold and **void your warranty.**

## Changing the Peri-Pump Dispense Cassette

Find instructions for physically installing the cassette in the Installation chapter or the cassette packaging.

### Change the Cassette Setting

It is critical that the instrument's cassette setting matches the installed cassette type. The instrument's setting is displayed in the main menu: CASS(#).

| Using the | Description |
|---|---|
| **Keypad** | 1. Press **Setup Menu**. (Setup Menu)<br>2. Select **PERI**.<br>3. Select **CASS**.<br>4. Select the matching setting.<br>5. Press **Main Menu** to verify the change. |
| **LHC** | 1. Select **Tools> Instrument Utilities**.<br>2. Select the **Peri-pump** tab.<br>3. Under **Cassette Type**, select the matching button.<br>4. Click Send. |

Take note of the **Cassette Requirement Mode** feature. It lets you require a specific cassette when defining a dispense step and offers three methods of handling a conflict with that requirement. When the cassette type setting does not match the required cassette, this feature provides a way to quickly manage or correct the conflict, including changing the cassette setting on-the-fly. See page 81.

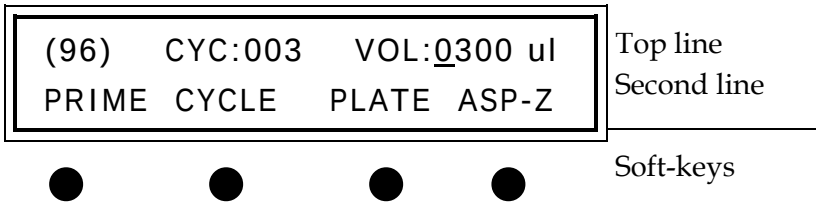
## Removing the Tubing Cassette

❖ Review the Tubing Cassette Diagram in the ***Installation*** chapter, if necessary, to recognize the names of the components.

1. Lift the **Tube Organizer** out of the fluid vessel.
2. Open the **Pump Cover**, moving it away from the pump.
3. Grasp with your left hand the **Tube Tensioner** attached to the pump's stainless steel plate as you use your right hand to release the latch on the right side of the pump (next to the caution symbol).
4. Still holding the tensioner with one hand, use the other hand to remove the **Center Holder** from its slot on the underside of the pump.
5. Hold both the Tube Tensioner and Center Holder in one hand while you slide the **Tip Holder** out of the dispense arm.

## WASH: Quick Wash (Keypad only)

Select **WASH** at the main menu to perform a quick wash. Alternatively, create a wash protocol as described on page 66.




❖ Quick Wash is not an option for 1536-well plates. You must define a protocol.

The EL406 saves the parameters that were last used to run a quick wash. They are displayed in the top-line of the LCD:

- **(96)** is the plate type. Select **PLATE** to change it.
- **CYC:003** is the number of wash cycles (one aspirate and dispense step per cycle). Select **CYCLE** to change the number of cycles to perform.
- **VOL:0300 ul** shows the dispense volume per well in microliters.

  
  
  To change the **dispense volume**, use the arrow keys to move the cursor to the desired number position. The cursor appears to underline a number: 0010. When the correct position is selected, use the number pad to enter the desired value.

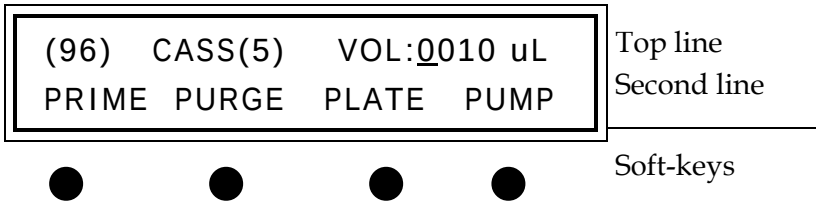
- **PRIME** to flush the tubing to remove air bubbles with the desired volume.
- **PLATE** lets you change the selected plate type and define a partial plate run, if applicable. See page 67.
- **ASP-Z** lets you change the height of the aspiration manifold above the wells.

When the desired values are entered, put a plate on the carrier and press **Start** to run the routine. Up to 10 quick wash routines are saved. When the maximum number has been defined, the newest replaces the oldest. Use the **Options** key to scroll the quick wash routines to select one.

If the quick wash routines are too limited to satisfy your assay requirements, use the LHC or the keypad to define a wash protocol. All wash parameters can be defined during protocol creation, including those recommended for cell washing, for example.

## DISP: Quick Dispense (Keypad only)

Select **DISP** at the main menu to perform a quick dispense.




The EL406 displays the last Quick Dispense that was run. With up to 3 dispensers available, you may first need to select the device:

- **PUMP** (when applicable) toggles through the dispensers, Peri-pump (displayed above), Syringe dispenser A and B. This option is not available when the Syringe dispenser module is not installed.
- **(96)** is the plate type. Select **PLATE** to change it.
- **CASS(#)** is the type of cassette. Make sure it matches the currently installed cassette. Learn how to change the setting on page 50. Refer to the EL406 Installation Guide or the cassette packaging for instructions on physically changing the cassette.
- **SYR-A** or **SYR-B** identifies the Syringe dispenser to be used in the Quick Dispense. Press **PUMP** to change the device selected. The cassette type (**CASS**) is displayed when the Peri-pump dispenser is selected.
- **VOL:0010 uL** shows the dispense volume (not the priming volume) per well in microliters (µL).

  
  
  To change the **dispense volume**, use the arrow keys to move the cursor to the desired number position. The cursor appears to underline a number: 0010. When the correct position is selected, use the number pad to enter the desired value.

- **Prime**: At the Peri-pump dispense screen, press and hold down prime key to prime the tubing. Fluid flows into the prime trough for as long as you press the key. For the Syringe dispensers, **PRIME** pumps 5000 µL each time it is selected.
- **Purge**: At the Peri-pump dispense screen, press and hold the Soft-key to purge the tubing. Fluid is pumped back into the supply vessel as long as you press the key.
- **Plate**: lets you to change the plate type and plate map or, more accurately, the columns of the plate to dispense to. Learn how on page 67.

When the desired values are entered, put a plate on the carrier and press **Start** to run the routine. Up to 10 quick dispense routines are saved for each dispenser. When the maximum number has been defined, the newest replaces the oldest. When the desired dispenser is displayed onscreen, press the **Options** key to scroll through the quick routines to select one.

## RUN: Running Pre-Defined Protocols

BioTek provides numerous pre-defined protocols for maintaining the instrument in top condition and for qualifying its performance. Review the list of pre-defined protocols on page 97.

To run a pre-defined protocol:

| Using the | Description |
|---|---|
| **Keypad** | 1. At the main menu, press **RUN**.<br>2. Press **Options** to scroll to the desired protocol or use the arrow and number keys to enter its number.<br>3. Press **ENTER** and follow the prompts. |
| **LHC**<br><br>Important: | 1.  Select **Open** and locate the EL406 folder.<br>2. Open the EL406 folder to access the Maintenance folder.<br>3. Select the desired protocol.<br>Be sure to customize the pre-defined protocols as described on page 75. |

## Creating Protocols: Washing, Aspirating and Dispensing Fluid

In addition to the quick routines available from the keypad's main menu, you can define and run protocols. Protocols offer more parameters, giving you the ability to fine-tune instrument performance, and perform more complex processing.

### Keypad Control

 Instructions for creating or modifying protocols using the keypad, as well as a map or grid showing the available options, can be found beginning on page 66.

### Liquid Handling Control™ (LHC) Software

 Launch the LHC software to create or modify protocols, wash plates, dispense fluid and so on. Turn to page 72 for an introduction to the LHC workspace.

 Select **Help>Help Topics** to learn about the LHC.
Watch the **Help>Tutorials** for an interactive learning experience.

## About Plate Types and Processing Patterns

With the required equipment installed, e.g. wash manifolds, the EL406 can process 96-, 384-, and 1536-well **Plate Types**.

The default parameters for wash and dispense steps represent the optimal positioning of the hardware for the plate type. The aspirate and dispense heights and horizontal positions can be adjusted when necessary for special situations.

### Processing Patterns

When using a 96-tube manifold to process a 96-well plate, all wells are processed simultaneously. But this is the only one-to-one relationship available with the EL406's hardware. To process 384- and 1536-well plates a processing pattern is needed. One advantage to this arrangement is the ability to process the plate partially, leaving some portions of the plate untouched. The processing pattern is determined by the hardware's footprint.

You can select which sectors, or columns to wash or dispense to, and which to skip. The graphic representation of the sectors/columns makes it easy to pick and choose which ones to process. Plate maps can be changed on-the-fly for both quick routines and protocols.

#### Plate Types Table

| | | Compatible Washers (# of Sectors) | | | Compatible Dispensers | | |
|---|---|---|---|---|---|---|---|
| **Plate Type** | **Columns x Rows** | **96-tube** | **192-tube** | **128-tube** | **8-tips (Peri)** | **16-tube** | **32-tube** |
| 96-well | 12x8 | √ (0) | N/A | N/A | √ | √ | N/A |
| 384-well | 24x16 | √ (4) | √ (2) | N/A | √ | √ | N/A |
| 1536-well | 48x32 | N/A | N/A | √ (12) | √ | N/A | √ |

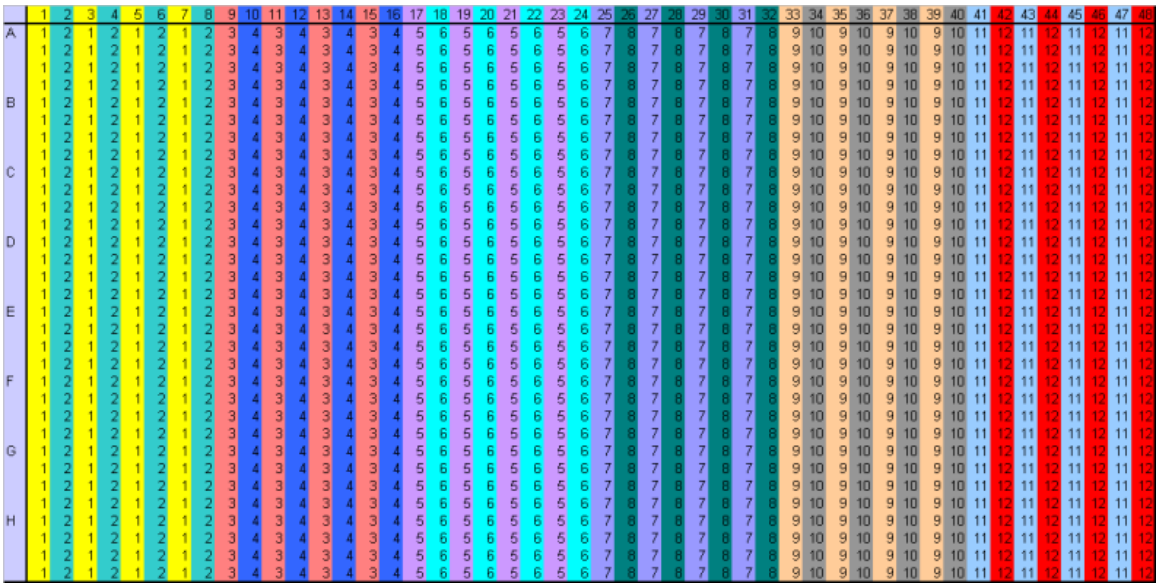
N/A = Not applicable, the plate type is not supported by the device.

#### 96-tube wash pattern for 384-well plate

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| B | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D |
| C | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D |
| E | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| F | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D |
| G | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| H | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D |
| I | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| J | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D |
| K | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| L | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D |
| M | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| N | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D |
| O | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| P | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D | C | D |

To process a partial plate, you must select the sector or sectors you want to process.

### 128-tube wash pattern for 1536-well plate



The 128-tube aspirate manifold processes 1536-well plates in 12 sectors. When you select the columns to process for a partial plate, they correspond to these sectors.

### 192-tube wash pattern for 384-well plate

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| C | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| D | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| E | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| F | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| G | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| H | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| I | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| J | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| K | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| L | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| M | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| N | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| O | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| P | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |

To process a partial plate, you must select the sector you want to process.

## How to change the plate type and plate map:

- Keypad control, see page 67.
- LHC control, see page 73.

## Operating with the BioStack

If you purchased BioTek's BioStack™ Microplate Stacker to operate with the EL406, here is some important information about running it:

**LHC Control:**

- The **LHC** lets you design protocols that integrate BioStack controls with EL406 steps. You must keep both instruments connected to the computer during operation to run these protocols.
- In the LHC, select **Help>Tutorials**, click **Sections** in the toolbar for a drop-down menu, select **Controlling the Bio-Stack with LHC**. It only takes a couple minutes to complete this interactive demo. It is a great way to learn about the special BioStack features offered with the LHC.

**Keypad Control:**

- The EL406's Quick Wash and Quick Dispense options do not function with the BioStack. You must create and **RUN** your own protocols to process plates using the BioStack.
- You can use the Quick Wash and Quick Dispense **prime** options. This is recommended especially prior to processing plates, to remove air from the tubing. Review the important information in the Maintenance chapter of operator's manual about running the pre-defined maintenance protocols when the BioStack is under keypad control.

### Install and configure the BioStack:

1. Set up the BioStack according to instructions in your ***BioStack Operator's Manual*** to interact with the EL406. Connect it to the:
    - Host computer (PC) when using the LHC to control the EL406.
    - Instrument when using the keypad to control the EL406.
2. Install the **BioStack™ PC Control Software** shipped with the stacker to control the BioStack using the LHC.
3. Align the BioStack's gripper with the EL406's plate carrier:

| Using the | Description |
|---|---|
| **Keypad** | 1. Press **Setup Menu**. (Setup Menu)<br>2. Select →<br>3. Select **BIOSTK**.<br>4. Select **ALIGN**.<br>Find instructions in your ***BioStack Operator's Manual***. |
| **LHC** | 1. Select **Tools> BioStack Utilities**.<br>2. Use the **Alignment Utility**.<br>Click the **Help** button for detailed instructions. |

4. Define the BioStack operating mode:

| Option | Description |
|---|---|
| **Keypad**<br>**Using the keypad to control the BioStack; stand alone operation** | 1. Press **Setup Menu**.  <br>2. Select →<br>3. Select **BIOSTK**.<br>4. Select **CONF**.<br>5. Select **BIOSTACK**. |
| **LHC** | **Important: When using LHC to control the EL406 and the BioStack:**<br>5. Select **MANUAL**, instead of **BIOSTACK**. |

❖ **To Restack or not?** Yes: to keep plates in the same order. No: to save time when the plate sequence is unimportant.

5. **Verify** the setup: perform a protocol with 1 or 2 plates specified in the LHC or placed in the input stack when under keypad control.

At the start of the day, power up the BioStack first, and then the EL406. Lift the BioStack's claw/gripper before turning it on.

# Optimize Performance

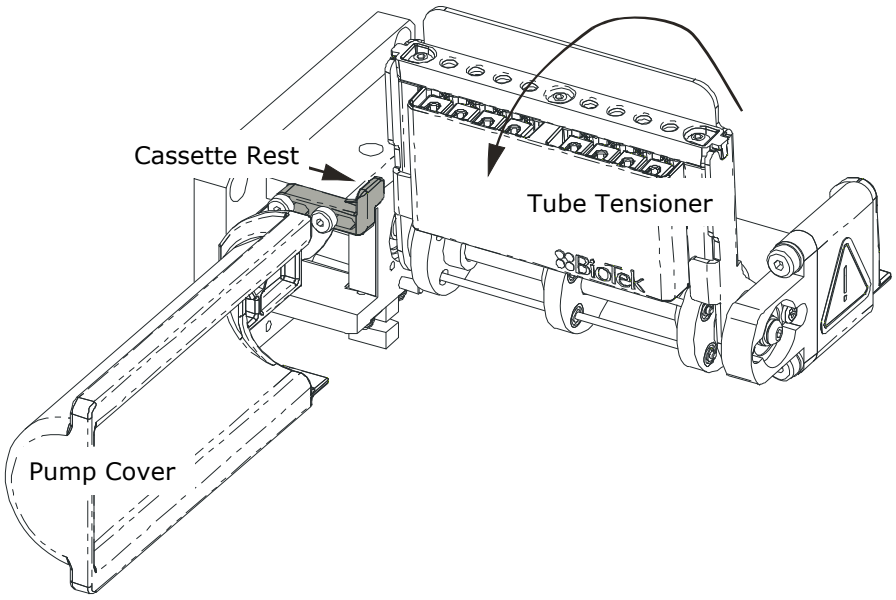
Here are some guidelines to ensure optimal performance and to prevent potential problems.

## Keep devices clean and tubing wet

The most critical factor for ensuring optimal performance is to adhere to the maintenance schedule described in the **Maintenance** chapter. Enable **AutoPrime** to keep tubes from clogging.

## Release the tension on the Dispense Cassette

When not in use, BioTek recommends releasing the tension on the cassette. This practice extends its life, preserving the tubing's integrity. To release the tension:




1. Open the **Pump Cover**.
2. Pull out the **Cassette Rest**.
3. Release the spring-loaded latch that holds in place the **Tube Tensioner** attached to the pump's stainless steel plate. It will rest against the cassette rest.

## Recommended volumes for fully priming EL406 devices

One of the best habits to develop is regular and frequent priming of the tubing and devices to keep them in a wetted state, free from clogs and air bubbles.

### *Washer*

To fully prime the wash manifold before processing plates, use these prime volumes:

| | **When tubes are empty** | | **When changing fluid** | |
|---|---|---|---|---|
| With Buffer Switching module | Prime Volume | Low Flow Path | Prime Volume | Low Flow Path |
| | 750 mL | 50 mL | 900 mL | 100 mL |

| | | | | |
|---|---|---|---|---|
| Without Buffer Switching module | Prime Volume | Low Flow Path | Prime Volume | Low Flow Path |
| | 360 mL | 50 mL | 400 mL | 100 mL |

❖ These prime values do not apply to 1536-well hardware which uses the Syringe module to dispense fluid.

### *Peri-pump dispenser*

**At the start of the day:**

Prime the tubing to prepare for a dispense run.

1 Reload the cassette and fill the supply vessel:

- When dispensing solutions not effected by water, simply prime with the dispense fluid.
- When dispensing protein solutions, first prime the tubing with a buffered saline solution to remove any traces of water in the tubing, then, prime with the dispense fluid.

2 Hold the **Prime** button on the keypad until fluid flows into the priming trough and all visible air bubbles have been removed.

**At the end of the day:**

Purge the tubing to reclaim the dispense fluid, then Prime the tubing to flush it clean.

1 Hold the **Purge** button on the keypad until the tubing appears empty.

2 Replace the supply vessel with the appropriate rinse fluid:

- When dispensing water soluble solutions, use DI water.

- When dispensing protein solutions, first prime the tubing with a buffered saline solution to remove protein particles, then, prime with DI water.

3 Hold the **Prime** button on the keypad:

- 1 µL cassette = 5 seconds
- 5 µL cassette = 7 seconds
- 10 µL cassette = 10 seconds.

### *Syringe dispensers*

The pre-defined maintenance protocols for the syringe dispensers fully prime the system, pumping 40 mL through the tubing and pump:

- S-DAY_RINSE_A&B – to fully prime both Syringe systems
- S-DAY_RINSE – defined for Syringe A only

Modify these protocols as needed when changing fluids and performing other tasks. For example, edit S-DAY_RINSE to use Syringe B or add another prime cycle when changing fluids to make sure all previously used fluid is expelled and replaced with the new liquid.

 The approximate dead volume for each Syringe dispenser system is 12 mL. Generally, three times the dead volume completely primes the system. When using precious fluids, e.g., expensive reagents, you can change the prime parameters: reduce the defined volume or number of cycles specified in the pre-defined protocols. Alternatively, create your own protocols.

## Best Practices for all EL406 Devices

- Fill the supply bottles with sufficient fluid. Make sure the supply tube is in the liquid.
- Make sure the bottles, solutions, and tubing are clean and do not contain any particles or mold. Water and dye solutions that are recycled over several days will grow algae, bacteria, molds, or other undesirable organisms.
- **Prime before dispensing.** Priming the tubing is the most critical factor in assuring optimal performance.
- Empty the waste bottles and firmly seat the bottles' caps and quick release connectors. To make sure fluid does not back up into the vacuum pump during operation keep the waste sensor cable installed and the waste detection sensor activated (see ***Sensors Enabled*** on page 86). If fluid collects in the overflow bottle, thoroughly rinse the level switch assembly and bottle.

- Put microplates on the carrier with position well A1 in the left rear corner as you face the instrument, and firmly seat the plate in the carrier.
- Rinse and soak the fluid path, clean and replace components, and decontaminate the instrument as described in the ***Maintenance*** chapter.

## Before *using the Washer*

- The supply tube should extend to the bottom of the bottle and be cut at the end (see drawing to the right) to ensure free flow of liquid at the bottom of the supply bottle.
- Check the external tubing connections for kinks and clogs.
- To avoid creating air bubbles every time the supply bottle is filled, make a mark halfway down the bottle and refill when the fluid level has dropped to that point. Unscrew the cap and let it hang over the side just enough to avoid emptying the inside tube and enough to refill the bottle.

## Before *using the Peri-pump*

- For top performance and to preserve precious fluids, **Purge** the fluid at the end of a dispense run and **Prime** the tubing before dispensing. The tubing is permeable to air. When 20 minutes or more have elapsed between dispenses, or less than 20 minutes when using 1 µL cassettes, it is important to thoroughly prime the tubing before dispensing.
- Use the **priming trough insert** to capture expensive reagents for reuse. The Peri-pump's insert can hold up to 12 mL.
- **Filter the dispense fluid** to 50 microns before dispensing with the 1 µL cassettes. The dispense tips are very small. Filtering the fluid helps prevent clogging.
- **Select the right cassette** for the job: match your desired dispense volume to the recommended cassette. The smallest recommended volume for a cassette type is one aliquot. An aliquot matches the cassette type, 1 µL for the 1 µL cassette, 5 µL for the 5 µL cassette, and 10 µL for 10 µL cassette.
- **Dedicate cassettes for specific fluids** or applications. Reserving specific cassettes for specific uses avoids contamination.
- When the dispenser is idle, **release the Tube Tensioner** element of the cassette from its place on the pump to minimize unnecessary stretching of the tubing. This is especially true for the 1 µL tubing. The best practice is to unload the 1 µL cassette when dispensing is completed.
- To more quickly dispense to **384-** and **1536-**well plates, use the Instrument Utilities to change the **Dispense Pattern** to **Row**. If precision is more important than speed, keep the pattern set to **Column**.

### Before *using the Syringe Dispenser*

- Sufficiently prime the Syringe dispenser to ensure precision and accuracy: **increase the number of prime cycles** to adequately remove all air bubbles from the tubing.
- Use the **priming trough insert** to capture expensive reagents for reuse. Each Syringe dispenser's insert can hold up to 6 ½ mL.

## Optimize protocols to improve evacuation

When a wash protocol leaves too much residual fluid in the wells, optimize the protocol with these recommendations:

- Add a secondary aspiration to the aspirate steps, including Final Aspirate,
- Decrease the aspirate Travel Rate,
- Add a Delay to the aspirate step and/or Final Aspirate,
- Lower the aspirate height (Z-axis position).

## Recommendations for 1536-well Hardware

When using the 128-tube aspirate manifold and the 32-tube Syringe dispenser manifolds, try these recommendations to achieve top performance. Experiment with the parameters and settings to determine the optimal values for your assays.

- **High-throughput** - to speed up processing try these settings:

| | |
|---|---|
| Plate Clear Height | 90 steps |
| Aspirate Travel Rate | 6 CW |
| Dispense Flow Rate | 5 |

  Use the **Adjust Utility** to test the Plate Clear Height before applying
- **Improve Accuracy**: set the Dispense Flow Rate to 2.
- **Improve evacuation**: for the smallest residuals use a standard Aspirate Travel Rate, not a CW (cell wash) rate.
- **Increase residuals**: to retain fluid in the wells, increase the aspirate height, Z Position. For example, in our testing, 56 steps, instead of 40, left 3 µL DI water in each well.

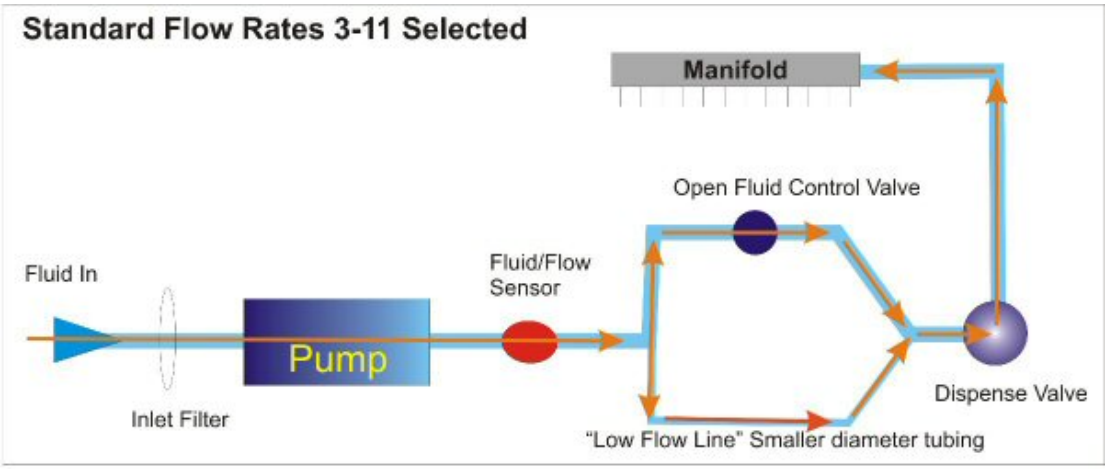
❖ Remember to keep the dispense manifolds wet when not in use: soak the tubes in the priming trough inserts with **AutoPrime**, for example.

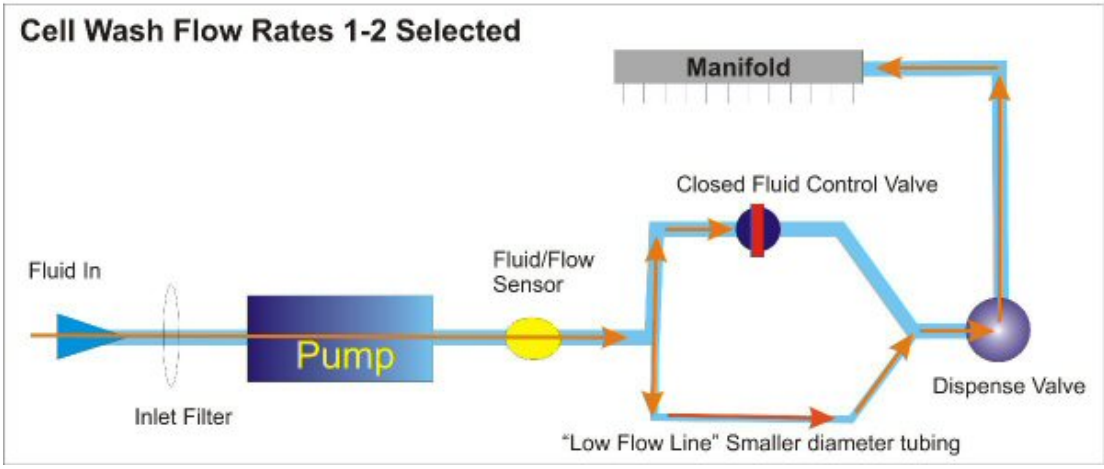
## Cell Wash Assays

❖ Find instructions for defining a cell wash protocol below.

### Low-flow Fluid Path

The EL406 supports cell-based assays that require the addition and removal of buffer solution without disrupting the cells in the wells of the microplate. Cells are often dislodged when fluid is dispensed at too high a pressure and lost during subsequent aspiration of the fluid from the well unless counter measures are taken. The EL406 is equipped with a low-flow fluid path that provides a "cell wash" alternative for cell-based assays.




The low-flow tubing is used during a wash step when the Flow Rate is set to 1 or 2. It dispenses fluid to the wells slowly enough to avoid damaging the cells. Note that the low flow line is always open, i.e. some fluid flows through the tubing during normal dispenses. For this reason, priming the low flow tubing is recommended for all Prime steps.

### Additional Techniques

**Delay Aspiration**: Also critical to cell-based assays is delaying aspiration to allow the slower dispense process to finish before beginning fluid removal from the well. This option, offered as part of the dispense step, is called **Delay Start of Vacuum** or **Vacuum Delay Vol** on the keypad.

**Adjust the Aspirate Travel Rate and Aspirate Height**: when defining the aspirate step select one of the specially designed travel rates that minimize turbulence in the wells. Also consider increasing the aspirate height to leave more residual fluid in the wells to protect the cell layer.

**Adjust the Dispense Flow Rate, Height and Position**: when defining the dispense step be sure to select one of the special Flow Rates that trigger use of the low-flow tubing, 1 or 2 CW. Also consider adjusting the dispense height and position to aim the fluid at the side of the well to further minimize turbulence.

❖ Use the **Adjust Utility** to determine the optimal height and position of the tubes when addressing the wells.

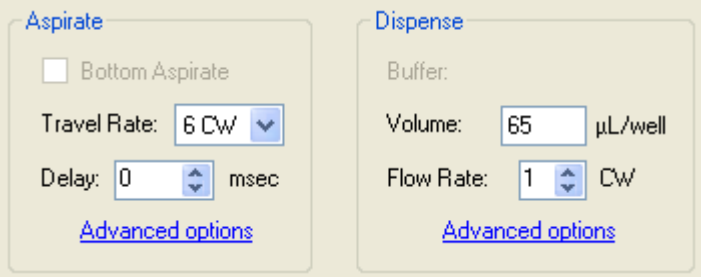
## Define a Cell Wash Protocol

Adjust the volumes recommended in this procedure to meet your specific needs. Also consider changing the dispense height and position to aim fluid at the side of the well to reduce turbulence and change the aspirate height to increase the amount of residual fluid left in the well to protect the cell layer.

1.  Create a new protocol.
2. Add a **Prime** step, especially when the lines are empty or when changing fluids. Recommended volumes in mL:

| | Regular Prime | | Changing Fluids | |
|---|---|---|---|---|
| | Prime | Low Flow | Prime | Low Flow |
| With Buffer Switching | 400 | 300 | 600 | 400 |
| No Buffer Switching | 200 | 150 | 300 | 200 |

3. Add a **Wash** step and define it as you normally would, except with these special parameters:
   - Set the Aspiration **Travel Rate** to **6 CW** and the **Delay** to **0**.
   - Set the Dispense **Flow Rate** to **1** or **2**.
   - When using the keypad set the **Vacuum Delay Vol**.




- **When using the LHC**: click the Dispense Advanced Options link and set the **Delay start of Vacuum** until sufficient fluid has been dispensed. For small dispense volumes, BioTek recommends setting the delay volume to equal your dispense volume.

❖ Optionally, change the dispense height and position to aim fluid at the side of the wells to reduce turbulence and change the aspirate height to increase the amount of residual fluid left in the well to protect the cell layer.

# Keypad Control of the EL406

This section provides a detailed description of how to use the keypad.

## Create or Edit a Protocol

At the main menu:

1 Select → for more options.

2 Select **DEFINE** and then, **CREATE** or **EDIT**.

| | **CREATE** | **EDIT** |
|---|---|---|
| 3. | **Name** the protocol (see page 69) and select the **Plate Type**. Press the Soft-key to scroll through options. Press **Enter** after making selections to proceed. | Select the protocol to edit: enter its number or use the **Options** key to scroll through the stored protocols to select one. Then, you can edit the name and plate type, if desired. Press **Enter** to proceed. |
| 4. | Select **ADD** to define the first step: then, select the device to use or **SHAKE** to mix or soak the plate's contents. | **EDIT** the first step or press the **Options** key to scroll to the step you want to change in a multi-step protocol. |

Select the device to use:

| | | |
|---|---|---|
| | **WASHR** | To use the wash manifold to wash, dispense or aspirate fluid or to run AutoClean. |
| | **PERIP** | To dispense fluid using the Peri-pump dispenser. |
| | **SYRNG** | To dispense fluid using one or both of the Syringe dispensers, A or B. |
| | **SHAKE** | To mix the contents of the plate and/or soak or steep the fluids for a specified time period. |

| | | |
|---|---|---|
| 5. | Select the action you want the device to perform and then define the step's parameters. Press **Enter** to proceed. | |
| 6. | **Keep Added Step?** | **Save Step Changes?** |
| | Select **Yes** or **No** using the Soft-keys to save or discard your inputs for the current step. | |
| * | Press **Main Menu** to end the session at any time. Then, select **RUN** and select the protocol to run it. | |

❖ See a description of the parameters for each step and their default values in the tables beginning on page 90.

## Define the Plate Type and Plate Map (or Partial Plate)

❖ Learn about the supported plate types and processing patterns on page 54.

The EL406, depending on the currently installed hardware, can process 96-well, 384-well, and 1536-well plate types. Washing and dispensing to a partial plate is limited to choosing the sectors to be processed when washing a 386- or 1536-well plate and the columns to dispense to when dispensing to any plate type. This requires defining a "plate map." By default, the whole plate is processed.

### Quick Routines: Quick Wash and Quick Dispense

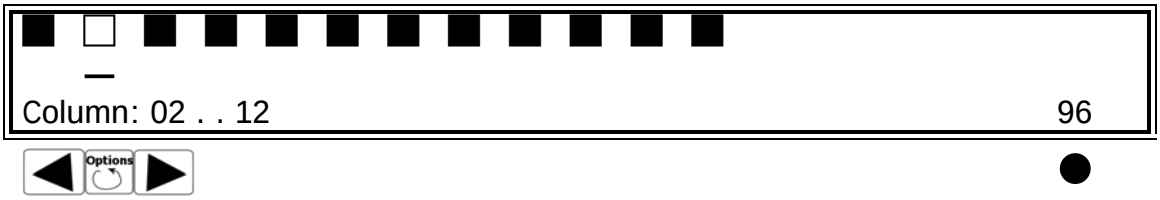
- When defining a quick routine, select **Plate** with the Soft-key to set the plate type, and to define the plate map.

### Protocols: Wash and Dispense

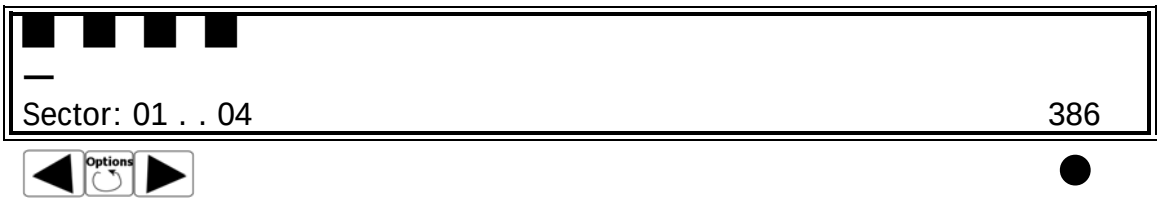
- When creating or editing a protocol, set the **Plate Type** after naming the protocol. Press the Soft-key to scroll to the correct plate type.
- To define the plate map, or more precisely, the columns or sectors to wash or dispense to:
  - Wash step: select **OPTS** and then **FORM**. First choose the processing mode Sector or Plate. Then, you will proceed to the plate map screen.
  - Dispense step: press Enter to proceed to the plate map screen.

### To change the plate type:

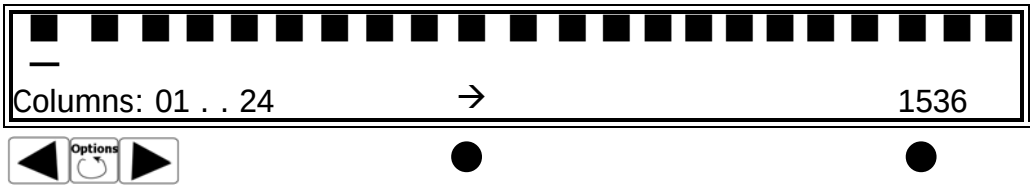
- Repeatedly press the Soft-key under the plate type (in the right corner of the display) to scroll through the available options. Hold the Shift key while pressing the button to reverse direction.




The two-line display changes to show a representation of the current plate type's columns or sectors in the top line. The display shows each column as a filled or empty square; empty columns/sectors will not be dispensed to or washed. For the current plate type, shown in the right corner, the left corner shows the selection range, beginning with the currently selected column/sector. (In the top example, column 2 is currently selected.)




Press the **Clear** button once to empty all the columns. Press it again to fill all the columns. This is useful when you want to dispense to only a couple columns.




- **1536-well plates** require two screens to show all 48 columns. Select the → key to toggle between the two screens to select the columns to dispense to.

**To change the plate map (selected columns):**

1. ◂ ▸ Use the arrow keys to move the cursor to the column/sector you want to change. The cursor underlines the currently selected column and its number is shown in the display. (In the top example, column 2 is currently selected.)
2. Press the **Options** key to toggle between filling the column or not. When the image of the column is filled it will be dispensed to. Conversely, when the column image is blank or unfilled, the column will not be dispensed to.
3. Press **Enter** to save the settings and continue.

## Common tasks using the keypad

This section contains instructions for commonly performed tasks.

How to:

- **Name a protocol**
- **Copy a protocol**
- **Use AutoClean to clean the wash manifold**
- **Add a Secondary Aspiration to a wash cycle**

Also see:

- Create or edit a protocol: see page 66.
- Run a pre-defined protocol: see page 53.
- Perform a Quick Wash or Quick Dispense: see page 51.

### *How to name a protocol*


At the **Name** screen when you are creating or editing a protocol, you can enter up to 16 alphanumeric characters to name the protocol:

- Press **Shift** + the number key for **A-H**, or scroll through the alphabet with the **Options** key for **A-Z**.
- Press **Shift + Options** to reverse direction.
- Use the arrow keys ◄ ► on either side of the **Options** key to move the cursor within the display.
- Press its Soft-key to add one of the four symbols (- % & _) in the display to the protocol name.
- Press **ENTER** when you are finished to store the protocol name.

❖ If the program name already exists, an Invalid Protocol Name message displays and you must enter a unique name.

### *Copy a protocol*

**→>DEFINE>COPY**

You can save significant time creating protocols by copying one with similar parameters.

1 Select → at the main menu.

2 Select **DEFINE** and then, **COPY**.

3 Select the protocol you want to copy: enter its number or use the **Options** key to scroll through the stored protocols, e.g. **S-DECON**. Press **Enter** to select it.

4 Assign a unique name to the protocol, e.g. **SB-DECON**, and press **ENTER**.

5 Select **YES** to copy.

6 Select **EDIT** and make the needed changes.

7 When all changes are made, select **EXIT**.

### *How to use AutoClean to clean the manifold*

**→>DEFINE>ADD** or **EDIT>WASHR>ACLEAN**

Before running AutoClean, the EL406's ultrasonic cleaner, review the safety information in the Maintenance chapter of the operator's manual.

To create an AutoClean protocol using the keypad:

1. Select → at the main menu.
2. Select **DEFINE** and then, **CREATE**.
3. Name the protocol, e.g. ACLEAN.
4. Press **Enter** past the plate type.
5. **Add** a **Prime** step to fully prime the tubing with the cleaning fluid (water): **WASHR>PRIME** and define the parameters. Use the recommended volumes for fully priming the tubing on page 59.
6. **Add** an AutoClean step: **WASHR>ACLEAN** and set the duration.
7. **Save** the steps and select Exit.

Fill the supply vessel with sufficient water or cleaning fluid before running the protocol.

**1536-well hardware**: The 128-tube aspirate manifold cannot completely evacuate the priming trough. When AutoClean is finished, you must manually remove the residual liquid using a paper towel or other method. Be sure to follow the manufacturer's handling instructions for the cleaning fluid you are using.

**32-tube dispense manifolds:** Also see the Maintenance chapter for instructions to use AutoClean to clean the hard-to-clean dispense manifolds.

### *How to Add a Secondary Aspiration to a Wash Cycle*

When too much residual is left in the wells during a wash cycle, you can add a secondary aspiration to remove it. Secondary aspiration is recommended for optimizing performance. It is not available for 1536-well plates.

1. Edit the Wash step or stand-alone Aspirate step: **Define**>**Edit**>select protocol, and if necessary press **Options** to scroll to the desired step.
2. Select **ASPIR** and press **Enter** until **Secondary Aspirate** is offered.
3. Select **YES** to perform a second or crosswise aspiration.
4. Optionally, adjust the position of the manifold as it addresses the wells by changing the X–, Y-, or Z-Positions.

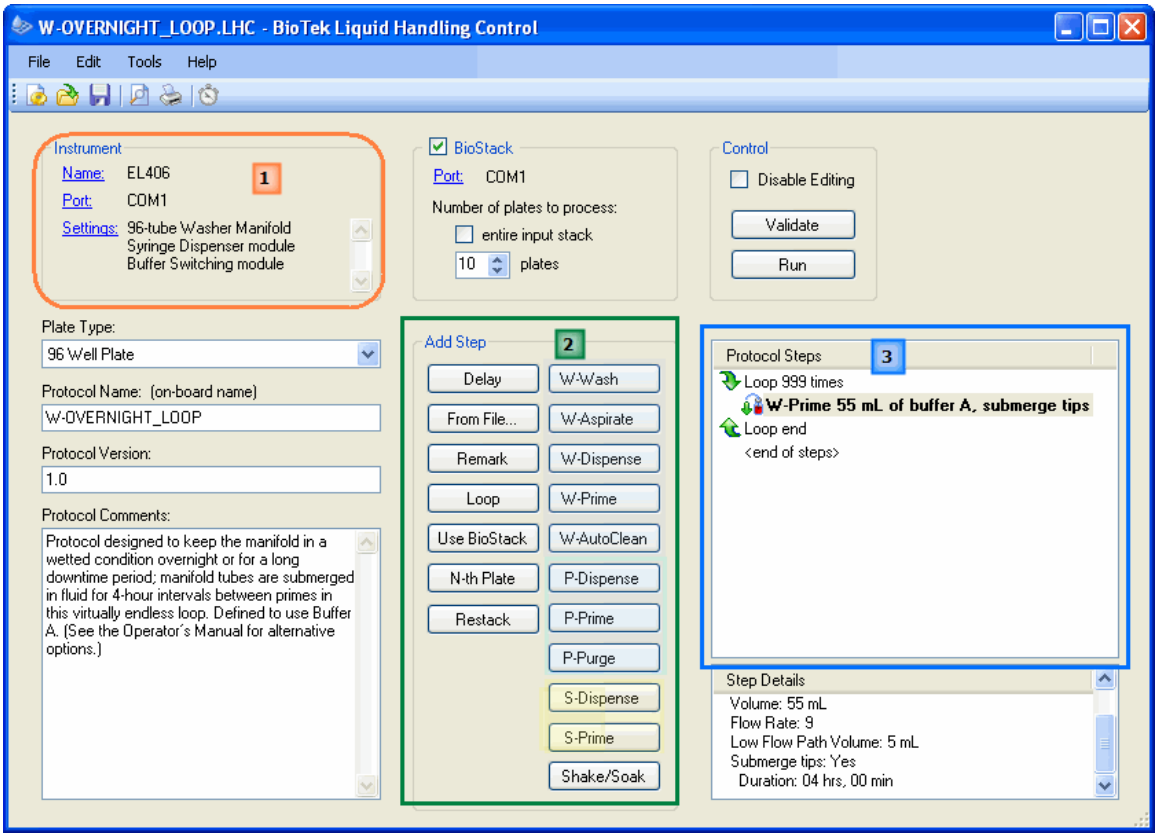
   To get hard-to-reach areas of certain types of wells, you may want to adjust the X- or Y- axes to better position the aspirate tubes. Or, you may want to lower the height by adjusting the Z-axis, to better evacuate fluid.

❖ Run the **Adjust Utility**: To determine the precise positioning of the dispense and aspirate tubes.

**Final Aspirate**: For the most complete evacuation of the wells, consider adding a Secondary Aspirate to the Final Aspirate: Select **OPTS>POST** at the Wash Step Parameters menu and follow the prompts to enable the feature.

# LHC Control of the EL406

This section provides an introduction to the LHC workspace for controlling the EL406. More information is available in the LHC Help system.




1 **Instrument Settings** described on page 78

2 **EL406 Steps** described below

3 **Protocol Definition** described on the next page

## EL406 Steps

Because the EL406 offers two or three devices in one instrument: a microplate washer, a peristaltic pump dispenser called the Peri-pump, and, optionally, dual Syringe dispensers, there are action buttons for each component. Click the action button and define the parameters to add that step to the protocol:

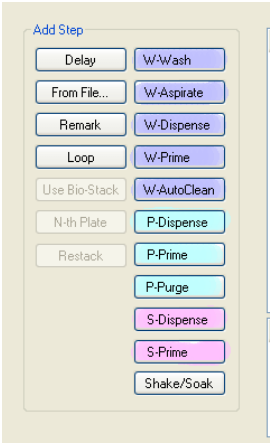
- **W-** for steps performed by the **Washer**.
- **P-** for steps performed by the **Peri-pump** dispenser.
- **S-** for steps performed by one of the **Syringe** dispensers, when installed.

- Shake/Soak Shake and soak are device-independent.

## Define a Protocol

Review "Define a Protocol" in the LHC's Help, if you haven't already done so.

In short:




1. Click a button in the Add Step area.
2. Define the parameters for the step in the dialog that opens.
3. Continue adding steps, if desired.
4. Save the file and/or click **Run** to execute the protocol.

Double-click a step in the protocol to open it for editing.

Highlight a step and press **Delete** to remove it.

Click and drag a step to change its sequence order.

❖ Learn details of each action step in the LHC's Help: select **Help>Help Topics**

### Run a protocol:

Run

After defining the protocol steps, make sure the device is properly prepared, e.g., supply bottle is filled and connected. Put the plate on the carrier and click **Run** when you're ready.

## Define Your Startup Preferences

You can save enormous time creating protocols by following these steps to define a **New Protocol** template and use it at startup.

### Create a protocol template

1. Click the **New** button or select **File>New**.
2. Click Name. Select the instrument if you have more than one, and define its Port and Settings.
3. Optionally, select the Plate Type, fill in the text fields, and add any steps that you want all new protocols to include.
4. Click **Save** and assign a unique name, e.g., Template.LHC.
5. Select **Tools>Preferences>New Protocol**.
6. Select the button for Protocol selected below to use as a template.

7 Click selected and select the protocol you created as a template.

**Define startup behavior:**

8 After completing the steps above, select the Startup Options tab.

9 Select the button for **New Protocol**.

10 Click **OK** to save your new preferences.

## Pre-Defined Protocols

BioTek provides pre-defined protocols for maintenance routines, quality control, and general samples for common applications like serial dilutions. Review a listing with brief descriptions of the **Pre-Defined Protocols** beginning on page 97.

Typically, you must **Customize the Pre-Defined Protocols** (see below) to match your instrument configuration and to meet your assay requirements.

**Instrument Settings**: In addition to action steps, every protocol file contains instrument settings (described on page 78), including COM port, manifold type, and so on. Edit the protocol to match your instrument's COM Port and other configuration details:

- **Power Users**: If you create protocols for multiple instruments or for other LHC users, read this more detailed description of how the EL406 validates a protocol to be run on a specific instrument.

❖ **Recommended**: After making changes to a pre-defined protocol, select **File>Save As** and give it a unique name. This practice preserves the protocol in the case of a future upgrade.

## File Location

The EL406 Interface Software (IS) installs the protocols in the Windows Common Applications Data Folder:

- Windows® XP, 2000: C:\Documents and Settings\All Users\Application Data\
- Windows® Vista™: C:\ProgramData\

The file location path continues:

[CommonAppDataFolder]**BioTek\Liquid Handling Control\Protocols\EL406**

Three folders are provided:

- **\Maintenance**: the recommended daily and periodic maintenance routines;
- **\QC**: some of the quality control or performance verification procedures;
- **\Samples**: examples of common applications, including washing 96- and 384-well plates, performing serial dilutions, and a cell wash protocol.

### Customize the Pre-Defined Protocols

BioTek provides pre-defined EL406 protocols for maintenance routines and quality control tests. You can quickly customize the protocols for regular use.

With the EL406 connected to and communicating with the host computer:

1.  Click the **Open** button, locate the EL406 folder and click **Open**.
2. Open the Maintenance or other folder and select the desired protocol.
3.  Change the COM port if necessary: click the Port link and use the drop-down list to select the port.
4.  Click the Settings link, which opens the Instrument Settings dialog.
5. Under Get settings from: click the instrument link.
6.  Click **Validate**.

   A "Validation successful" message is displayed unless the protocol cannot be run on your instrument.
7.  Save the protocol.

## Maintenance Protocols for the EL406

Regularly running maintenance protocols is required to keep the instrument in top condition.

Unless you have changed the file location:

1.  Click the **Open** button on the toolbar and locate the EL406 folder.
2. Open the EL406 folder to access the Maintenance folder.
3. Select the desired protocol.

### About the Maintenance Protocols


**File names**: The protocol file names mirror the LHC™ buttons. The prefix letter indicates the applicable device. For example, W-RINSE_&_SOAK is a daily routine for the washer component, and P-1UL_CASS_RINSE is designed for the Peri-pump.

Some washer protocols are further distinguished with a number that represents the 96- or 192-tube manifolds. Syringe dispenser (S-) protocols are defined for Syringe A only. Copy the protocol and modify it to perform the routine for Syringe B.

## Upload-Download Protocols

The LHC lets you transfer protocols from your computer to your instrument and back again.

***Limitation***: Protocols must contain only instrument-supported action steps to qualify for download. That is, the protocol cannot contain any of the LHC provided steps like Delay and Loop (buttons in the left column of the **Add Step** box of the main view). And, a **Protocol Name** is required.

❖ The instrument's main menu must be displayed for the LHC to communicate with it.

1. Select **Tools>Transfer Protocols**.
2. Make sure the desired protocols are displayed: check the Protocol Folder path for This computer. Refresh the list of protocols onboard the Instrument by clicking the Settings link.
3. Highlight one or more protocols in a display box. (Hold the Ctrl or Shift key to simultaneously select multiple files.)
4. Optionally, at the top left corner of the screen, choose to Disable Editing of transferred protocols to lock the protocols from editing or deleting when they are onboard the instrument.
5. Click the applicable **Upload** or **Download** button.

The LHC will confirm the transfer or prompt you for more information. When the transfer is complete, you can manipulate the files as you normally would in their new location.

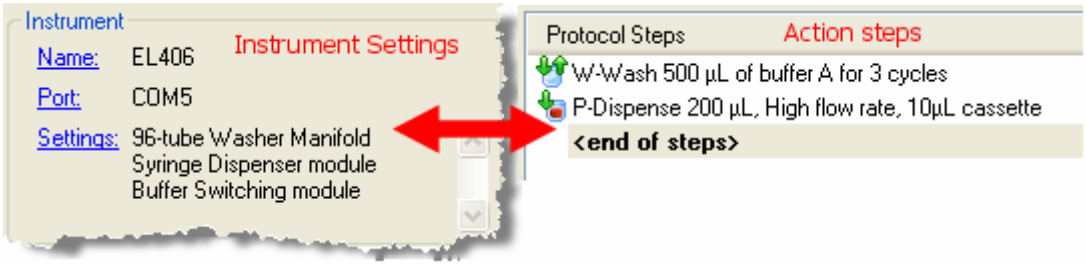
# LHC Protocols for the EL406 Explained

## Prerequisite

This discussion of the EL406 protocols will be easier to follow if you are already familiar with the LHC. Read "Understanding the LHC." You can search for it after selecting **Help > Help Topics**.

## Protocol Files

In addition to action steps with specific, user-defined parameters, each protocol file contains "Instrument Settings."




Generally, especially when you are managing only one instrument, the recommended best practice is to match the **Instrument Settings** to your instrument. However, unless a step requires a specific component for successful completion, the LHC runs the protocol even when the settings do not match the physical configuration of the instrument.

For example, a protocol whose Instrument Settings include Buffer Switching can be run by an instrument without the Buffer Switching module when none of the steps actually call for different buffer valves, i.e., all steps use the same buffer.

Similarly, an EL406 that does not have the Syringe dispenser installed can run protocols with Instrument Settings that include the Syringe, as long as the protocol does not include any Syringe dispenser steps.

This flexibility is useful when you are designing protocols for multiple instruments. It also makes it easier to design protocols when the computer is not connected to an instrument. And, it facilitates shipping Pre-Defined Protocols with the software.

## Validate versus Run

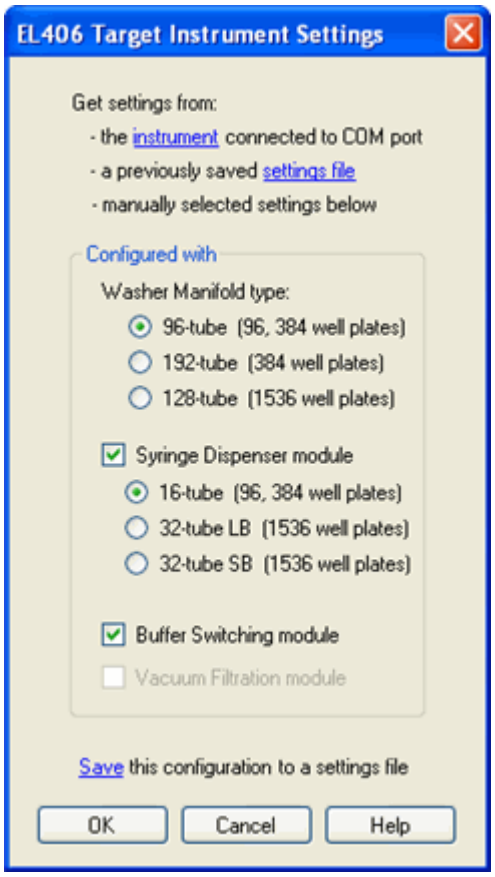

**Validate** checks the action steps against the protocol's Instrument Settings.


**Run** talks to the instrument to check the action steps against the instrument's onboard settings.

**Validate** will catch errors when the **Instrument Settings** have been changed after the protocol steps have been defined and there is a mismatch. **Run** performs a similar validation before executing the protocol. Errors are not reported unless the steps cannot be performed.

## Instrument Settings

**Click the Settings: link in the main workspace to open the dialog.**




Identify the exact configuration of your EL406 to ensure it can successfully perform the wash and dispense protocols created with the LHC.

Get settings from:

- Instrument: BioTek configures and tests the EL406 at the factory before shipping it. If you have not changed the instrument's onboard settings, you can safely click the instrument link to upload the correct settings from the instrument.
- Settings file: If you have previously saved the instrument's settings to a file (using the option at the bottom of the screen), click this link to import them.

**Configured with**:

Fill the checkboxes and buttons to select the currently installed manifolds and accessories:

❖ Be sure to complete all the steps for *Changing the Manifold*, when applicable, as described in ***Appendix B***.

### Save Settings File

If you have multiple instruments or use one instrument in multiple configurations, you can create unique settings files for each configuration and save time when defining protocols for that configuration.

Click the Save link and use Windows' file-saving dialog to create a .SET file based on the currently-selected parameters. Then use the settings file link under "Get settings from" to load the parameters.

# EL406 Settings and Utilities

BioTek configures the EL406's internal basecode with the correct settings before it leaves the factory. You can review the settings at any time and alter them when necessary. For example, if you change a hardware component, like the wash manifold, you must update the instrument's settings to match the hardware. You can also enable and disable sensors, AutoPrime, and perform other functions.

| Keypad | LHC |
|---|---|
| Use the keypad's **Setup Menu** button to access the utilities and to change settings. | Select **Tools> Instrument Utilities** to access utilities and to change settings. |

Setup Menu

## Setup Menu (Keypad Only)

Press the **Setup Menu** button in the middle of the keypad:

| WASH | PERI | SYR → | | TESTS | ADJST (Adjust Utility) | BIOSTK → | | AUTPRM | SNSRS (Sensors) | VACDIS |
|---|---|---|---|---|---|---|---|---|---|---|
| Manifold type | Purge | Manifold type | | Self Check | Plate Type: 96, 386, 1536 | CONF (Mode) | | Interval ↓ | VACUUM | Vacuum Dissipation |
| Plate clear height | Cass type | | | CHecKSUM: UI, MC, BioStack | Washer Aspir/Disp Axis: Man POS: CARX CARY | ALIGN | | Washer<br>Volume, Rate, Buffer, Submerge | WASTE | Delay in seconds |
| Park for shipping | Prime | | | | PeriPump First/Last col. Axis: Disp Pos: CarX/CarY | VERIFY | | Syringe A<br>Volume, Rate, Submerge | FLUID | |
| | Pattern: Row or Column | | | | Syr-A/Syr-B First/Last col. Axis: Disp Pos: CarX CarY | | | Syringe B<br>Volume, Rate, Submerge | FLOW | |
| | Mode: Prompt, Error, Set | | | | | | | | | |

## Washer Utilities and Settings

Define your instrument's configuration and other details for optimal operation. Also see **AutoPrime** and **Sensors** in this section.

### Manifold Selection

| Using the | Description |
|---|---|
| **Keypad** | 1. Press **Setup Menu**.<br>2. Select **WASH** and then **MAN**.<br>3. Select the currently installed wash manifold by pressing its Soft-key: 96-, 192-, or 128-tube. |
| **LHC** | 1. Select **Tools> Instrument Utilities**. |

| | |
|---|---|
| | 2. Select the **Washer** tab.<br>3. Under Manifold Selection, choose the button that represents the installed manifold.<br>4. Click Send to send this setting to the EL406. |

### Changing the Washer Manifold

BioTek offers a second washer manifold as an accessory to the instrument. Follow the instructions provided in ***Appendix B*** to physically change the manifold and to reconfigure the instrument to function properly with the installed manifold.

## Plate Clearance Height

To accommodate plates that are slightly taller than standard plates, use this setting to make sure the manifold tubes rise high enough above the plate to prevent crashes when the plate carrier moves. Default setting: 130 steps or 16.52 mm.

First use the **Adjust Utility** to determine the correct setting.

| Using the | Description |
|---|---|
| **Keypad** | 1. Press **Setup Menu**.<br>2. Select **WASH** and then **CLRHT**.<br>3. Enter the number of steps determined using the Adjust Utility. |
| **LHC** | 1. Select **Tools> Instrument Utilities**.<br>2. Select the **Washer** tab.<br>3. Under Plate Clear Height, Enter the number of steps determined using the Adjust Utility.<br>4. Click Send to send this setting to the EL406. |

**1536-well hardware**: Lowering the Plate Clear Height to 90 steps for the 128-tube aspirate manifold sped up processing in tests performed by BioTek. Use the Adjust Utility to verify this height is compatible with your plates.

## Position Manifold for Shipping

Prior to packing up the EL406 for shipping, you must position the Washer to allow installation of the shipping bracket.

| Using the | Description |
|---|---|
| **Keypad** | 1. Press **Setup Menu**.<br>2. Select **WASH** and then **PARK**. |
| **LHC** | 1. Select **Tools> Instrument Utilities**.<br>2. Select the **Washer** tab.<br>3. Under Service Functions, click the shipping bracket link. |

## Peri-pump Utilities and Settings

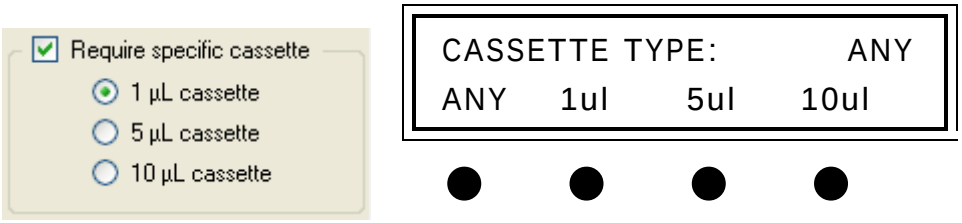
When using the keypad, the Setup Menu for the Peri-pump includes basic functionality like priming and changing the cassette setting; see page 50.

### Dispense Pattern

When processing 384- and 1536-well plates, select the fill pattern: by column or by row. For these high-density plates, the 8-tip manifold must address the plate multiple times to fill it. Column-wise dispensing fills each column before moving to the next. Row-wise dispensing fills the first 8 rows, then reverses direction to fill the next 8 rows, and so on. Choose row for faster throughput. Column is the default setting because it is more precise. Once it is defined, your **Pattern** preference will apply to all runs, Quick Dispenses and Protocols.

| Using the | Description |
|---|---|
| **Keypad** | 1. Press **Setup Menu**.<br>2. Select **PERI** and then →.<br>3. Select **PATRN** and then **COL** or **ROW**. |
| **LHC** | 1. Select **Tools> Instrument Utilities**.<br>2. Select the **Peri-pump** tab.<br>3. Under Dispense Pattern, select by column or by row.<br>4. Click the Send link. |

### Cassette Requirement Mode




When defining a Peri-pump dispense step, you can require a specific cassette type be used or not.

When defining a Peri-pump dispense step, you can require a specific cassette type or allow any cassette type to be used. When a specific cassette type is required, the EL406 needs to know how to behave when the required cassette is not installed at runtime:

- **Prompt** the user to confirm that the required cassette type is installed. This option provides the best protection against an unintentional mismatch. If the cassette type itself has been physically changed to match the protocol, but the instrument's setting has not been updated, it will update the EL406's setting upon confirmation. However, if the cassette has not been changed to match the protocol, users are given a chance to cancel the run, fix the error, and rerun the protocol.

- Return an **Error** code and stop processing. This option gives robotics programmers the ability to design and run unattended processing routines without fear of a message screen interrupting the operation.
- Automatically **Set** the cassette type and continue the routine without notifying the user. This option changes the EL406's setting without a confirmation. This option is for advanced users only.

❖ No action is taken when the cassette type setting matches the protocol's required cassette type.

| Using the | Description |
|---|---|
| **Keypad** | 1. Press **Setup Menu**.<br>2. Select **PERI** and then →.<br>3. Select **MODE** and then **Prompt** or **Error** or **Set**. |
| **LHC** | 5. Select **Tools> Instrument Utilities**.<br>6. Select the **Peri-pump** tab.<br>7. Under Cassette Requirement Mode, select an option.<br>8. Click the Send link. |

## Syringe Dispenser Utilities and Settings

Three models of dispense manifold are available for the EL406's Syringe dispenser module. You must define your instrument's configuration and other details for optimal operation.

### Manifold Selection

| Using the | Description |
|---|---|
| **Keypad** | 1. Press **Setup Menu**.<br>2. Select **SYR** and then **MAN**.<br>3. Select the currently installed dispense manifold by pressing its Soft-key: 16, 32LB (large bore) or 32SB (small bore). |
| **LHC** | 1. Select **Tools> Instrument Utilities**.<br>2. Select the **Syringe Dispenser** tab.<br>3. Under Dispenser Assembly, choose the button that represents the installed manifold.<br>4. Click Send to send this setting to the EL406. |

The EL406 Interface Software (IS) is required to update the instrument with additional configuration data for the Syringe dispensers. You must be controlling the EL406 with the LHC software or obtain a copy of the EL406 IS Utility from BioTek's customer resource center: https://customer.biotek.com.

# Adjust Utility

## About the Adjust Utility

The Adjust Utility is a tool for determining the precise positioning of the dispense and aspirate tubes when addressing the plate. Generally, the default positions function perfectly, but certain assays may be improved by repositioning the dispense or aspirate tubes. For example, to minimize cell damage, tubes can be positioned at the sides of the wells, rather than the center. Use the utility to identify the offsets required, then, enter these offsets when defining the wash or dispense step.

The Adjust Utility puts the plate in its run position and lets you move the washer's manifold, the Peri-pump's tip holder or the Syringe dispenser manifolds step-by-step to the desired position. You can:

- Raise or lower the tubes/dispense tips in the Z-axis to determine the desired dispense or aspirate height. BioTek has set the default position for each plate type based on optimal performance.

Positioning the tubes in the X and Y axes occurs in relation to the center of the well and is accomplished by moving the plate carrier. The default position is the center of the well, 0 steps. Negative offsets are left and back of center; positive offsets are right and forward of center.

- Move the carrier left or right in the X-axis.
- Move the carrier forward and back in the Y-axis; only available for the washer manifold.

Warning: The Adjust Utility does not have limits to protect you from making bad choices. It is possible to identify dispense positions that miss the wells, for example. If incorrect values are defined in the protocol, you'll have a big mess on your hands.

## How to use the Adjust Utility (Using the LHC)

1. Select **Tools>Instrument Utilities>General Settings**.
2. Click the Adjust Utility link.
3. Click the **Help** button for guidance.

## How to use the Adjust Utility (Using the Keypad)

1. Place a microplate on the carrier.
2. Press the **Setup Menu** button.
3. At the Setup menu, select → for more options and then select **ADJST**.
4. Select the **PLATE TYPE**: 96, 384 or 1536 and press **Enter**.
5. Select the device: Washer, Peri-pump, or one of the Syringes.

| Washer ↓ | | Dispensers: Peri-Pump, Syringe A or Syringe B ↓ | |
|---|---|---|---|
| **Aspirate** | **Dispense** | **First column** | **Last column** |
| ↓ | | ↓ | |
| **MAN** | Manifold Z-axis (up/down) | **DISP** | Manifold Z-axis (up/down) |
| **CARX** | Plate carrier X-axis (left/right) | **CARX** | Plate carrier X-axis (left/right) |
| **CARY** | Plate carrier Y-axis (front/back) | **CARY** | Plate carrier Y-axis (front/back) |

6 After selecting the device, select a run position: **ASPIR** (aspiration), **DISP** (dispense), or **FIRST COLUMN** or **LAST COLUMN**. Only one position may be viewed at a time.

7 At the **AXIS** screen, choose an axis, Z, X or Y. The top line of the display indicates which axis is active, and the offset position of that axis.

8 Closely observe the position of the hardware. Press the ◄ (reverse) key to single-step the offset in a negative direction, and the ► (forward) key to single-step the offset in a positive direction.

9 When the desired offset position is found, record the position number for later use when defining a wash, dispense, or aspirate step.

10 To quit the Adjust Utility, press **Main Menu**. The carrier and manifolds return to their default positions.

## BioStack Settings and Utilities (Keypad only)

**Setup Menu > → > BIOSTACK**

From the Setup Menu, select **BIOSTK** to set the EL406 to operate with the Stacker:

- **CONF** (Configuration): to set the mode to BioStack or Manual.
- **ALIGN** (Alignment): to use the BioStack's alignment utility to align the robot's wrist to deliver and retrieve plates.
- **VERIFY** (Verification): to test the accuracy of the BioStack-instrument interface.

Refer to the BioStack Operator's Manual for detailed instructions.

❖ **Note**: When using the LHC to control the Stacker, set the configuration mode to **Manual** and use the **Tools>BioStack Utilities** menu options instead of the keypad.

## AutoPrime

❖ Learn about **AutoPrime™** and how to enable it on page 88.

## Sensors

The EL406 is equipped with four sensors to facilitate operation and prevent problems:

- **Vacuum Detection**: verifies sufficient vacuum to function properly.
- **Waste Detection**: monitors fluid level in waste bottles before beginning a run. Make sure the waste sensor cable is plugged into the correct port.
- **Fluid Detection**: verifies sufficient fluid level to begin and at completion of the run. Washer must be primed to fill the tubes prior to a run. An error at the end of the run indicates an insufficient volume of fluid was dispensed.
- **Flow Detection**: monitors fluid flow during the run. Issues a warning when the pump is interrupted. An error indicates a problem with the pump.

| Using the | Description |
|---|---|
| **Keypad** | 1. Press **Setup Menu** and select → twice.<br>2. Select **SNSRS**.<br>3. Enable or disable each sensor individually: VAC, WASTE, FLUID, and FLOW. |
| **LHC** | 1. Select **Tools> Instrument Utilities**.<br>2. On the General Settings tab, fill or empty the checkboxes to enable or disable Vacuum and Waste detection. Click the Send link to execute a change.<br>3. Select the **Washer** tab for Fluid and Flow detection. Click the Send link to execute a change. |

When the sensors are triggered, the LHC or the keypad will display an explicit message describing the failure or required action.

❖ BioTek recommends keeping the detection systems activated. One exception is when running very low density fluids like alcohol. If doing so causes errors, deactivate the **Fluid** sensor while using these types of fluids.

## Vacuum Dissipation

**About Vacuum Dissipation Delay**: Some equipment requires a delay at the end of a run to allow air into the fluid containers to prevent the vacuum pump from overworking. A delay of 1 second/liter has proven to be reliable in preventing certain pumps from blowing an auxiliary 5-amp fuse (PN 46055). When necessary, specify a delay of 10 seconds when using a 10-liter bottle, 20 seconds when using a 20-liter bottle, and so on.

**Delay**: 5 second default value; valid range is 0-50 seconds.

- For high-throughput plate processing, when using a BioStack and a high flow pump, for example, eliminate the delay: set the value to 0 seconds. For

standalone use, BioTek does not recommend changing the default setting, unless you are troubleshooting.

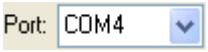
| Using the | Description |
|---|---|
| **Keypad** | 1. Press **Setup Menu** and select → twice.<br>2. Select **VACDIS**.<br>3. Enter between 0 and 50 seconds to delay processing.<br>4. Press ENTER to exit and return to the menu. |
| **LHC** | 1. Select **Tools> Instrument Utilities**.<br>2. On the General Settings tab, define the **Delay** under Vacuum Dissipate.<br>3. Click the Send link to execute a change. |

## Downloading Basecode — EL406 IS Utilities Viewer

Before shipping the instrument, BioTek loads the EL406 with the latest basecode and configuration settings specific to your instrument. It may be necessary, however, to update the basecode at a later date. If this is necessary, contact BioTek TAC to obtain a copy of the latest basecode file.

**LHC users**: select **Tools> Instrument Utilities**

**Keypad users**: in addition to obtaining the basecode file, you must install the EL406 IS Utilities Viewer, and connect the instrument to your PC.

1 Define the COM port: 

The LHC identifies and lists the available physical and virtual COM ports.

2 Retrieve All onboard settings: Click the link to get all the instrument's settings simultaneously. It communicates with the EL406 and updates the information displayed in this dialog to reflect the instrument's onboard settings.

3 Under Service Functions click the Download Basecode link and **Browse** to the basecode file. Follow the onscreen prompts.

# AutoPrime

## About AutoPrime

The EL406 supports an AutoPrime option that automatically primes the washer and the dual syringes, if present, whenever the instrument is idle for a specified time. AutoPrime keeps the tubes wet between dispenses and other processes. And, AutoPrime's submerge feature provides a method of cleaning the aspirate and dispense tubes.

You can specify the downtime interval for all components and set the parameters for each component. Devices are primed consecutively, beginning with the syringe dispensers, not concurrently.

- Any interaction with the instrument resets the AutoPrime interval clock, including communication from the LHC. **The instrument must be at its main menu to run AutoPrime.**

## Turn On AutoPrime

**Set the AutoPrime Interval and Parameters:**

| Using the | Description |
|---|---|
| **Keypad** | 1. Press **Setup Menu** and select → twice.<br>2. Select **AUTPRM**.<br>3. Specify the idle-time interval to trigger AutoPrime: up to 24 hours in minutes and press **Enter**.<br>4. At the AutoPrime setup menu, select the device you want to enable: the **Washer** and/or one of the **Syringe dispensers**.<br>5. For each device, define the AutoPrime parameters: rate, volume, and buffer valve, if applicable. Press **Enter** at each screen to advance to the next.<br>Parameters match the device's regular prime step. Enter a time period to enable the submerge feature. |
| **LHC** | 1. Select **Tools> Instrument Utilities**.<br>2. On the General Settings tab, under AutoPrime Interval, use the spin buttons or enter the downtime interval to trigger AutoPrime.<br>3. Select the **Washer** tab and fill the checkbox to enable AutoPrime. Specify the priming parameters: rate, volume, and buffer valve, if applicable, and submerge duration, if any.<br>4. Select the **Syringe Dispenser** tab and do the same, if desired, to enable AutoPrime for these devices.<br>5. Click the Send link to execute a change. |

- Press the **STOP** button on the keypad to interrupt the AutoPrime routine when it is underway.

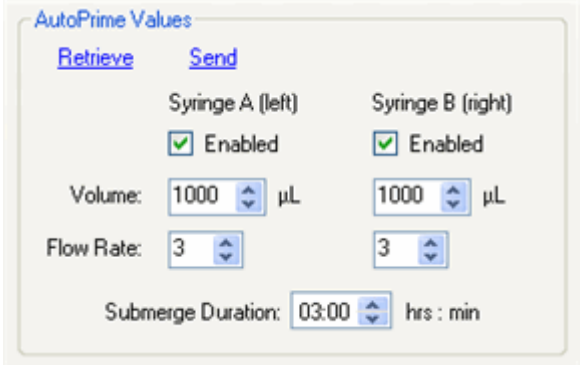
### Stop AutoPrime

The keypad displays a message, AutoPrime Running, when it is underway. Press the **Stop** button on the keypad to end the current session. To permanently stop AutoPrime from running follow the instructions referenced above, but disable, rather than enable it.

## Use AutoPrime to clean the Syringe Dispenser Manifolds

- Be sure to use the priming trough inserts when the AutoPrime submerge feature is enabled.

It is especially important when using the 32-tube SB dispense manifolds to keep the tubes wet to prevent clogs. One option is to modify the AutoPrime parameters used for idle periods during a regular work day to soak the tubing for longer periods overnight and on the weekends.




Define the AutoPrime values to fill the priming-trough-inserts and then submerge the tubes for several hours.

When the EL406 is set up for 1536-well washing, you can disable AutoPrime for the washer; the 128-tube aspirate manifold does not require the same level of soaking as dispense manifolds.

# Step Parameter Reference Tables

## Wash Step Parameters

→>**DEFINE**>**ADD** or **EDIT**>**WASHR**>**WASH** (keypad path)

Minimally, a wash step includes an aspirate step followed by a dispense step. Select and define each option to customize the parameters for your assay.

| Keypad name | Option | Description / Values range | Default values |
|---|---|---|---|
| CYCLES | **Cycles** | Each wash cycle first aspirates and then dispenses fluid to and from the plate. | 3 |
| ASPIR | **Aspirate** | | |
| | Travel Rate: | The rate at which the washer manifold travels down into the wells. The selection range is 1 to 5 for non-cell-based assays, from slowest to fastest. With these rates, the tubes slow their descent as they approach the defined aspirate height (Z Position) to aid complete evacuation of the well.<br>For delicate, cell-based assays, the range is 1CW (cell wash) to 4CW and 6CW. These rates minimize turbulence in the wells. The tubes descend at a constant rate to the specified height. Rate 6CW creates the least disturbance and performs fastest. | 3 |
| | Delay: | Amount of time the tubes stay at the aspirate height before lifting out of the wells. Define a delay between 0 - 5000 ms. Increasing the delay may improve evacuation of the wells. | 0 |
| | Positioning: | X- and Y- horizontal axes, Z- height (vertical) axis can be adjusted to improve performance. Default Z-axis for 1536-well plates is 40 steps. | Z = 30<br>X & Y = 0 |
| | Secondary aspirate: (except 128-tube for 1536-well plates) | Also called Crosswise Aspiration. First the wells are aspirated using the position defined above. The aspirate tubes rise and then descend to the secondary position to aspirate again. This option is not available for 1536-well plates. | No |
| DISP | **Dispense** | | |
| | Flow Rate:<br>96-tube & 192-tube manifolds | The rate at which the fluid is dispensed from the tubes. For cell-based assays, use rate 1 or 2 for gentle washing with the 96-tube manifold only. For normal dispensing, the range is 3-11, 3 is slowest and 11 is fastest. | 7 |
| | 32-tube dispenser: | For 1536-well plate processing, flow rates 1-5. | 3 |
| | Volume: | µL/well dispensed range:<br>▪ 96-tube manifold: 50-3000<br>▪ 192-tube manifold: 25-3000<br>▪ 1536-well (32-tube dispenser): 03-3000 | µL/well<br>96 = 300<br>384 = 100<br>1536 = 10 |

| Keypad name | Option | Description / Values range | Default values |
|---|---|---|---|
| | Buffer: | Buffer bottle selection. | A |
| | Positioning: | X- and Y- horizontal axes, Z- height (vertical) axis can be adjusted to improve performance. | Z = 120 |
| | Vacuum Delay | Suspends the vacuum pump until a certain volume is dispensed. This feature is applicable to cell wash operations only. It delays normal aspiration until the specified volume has been dispensed to the wells. The range is 10 to 350 µL/well. | 10 |
| OPTS | **Options** | | |
| | PRE | Pre-wash options: Pre-dispense, Bottom wash (if applicable) | |
| | MIDCYC | Mid-cycle options: Shake, Soak, Pre-dispense between cycles, | |
| | POST | Post-wash option: Final Aspirate | |
| | FORM | Format for washing 384- and 1536-well plates: Plate or Sector | |
| PRE | Pre-wash | Actions performed before the wash cycles begin | |
| | **Pre-dispense** | Quick, small prime to condition the tips before dispensing. | |
| | Flow Rate: | 96-tube & 192-tube manifolds: The range is 3-11, 3 is slowest and 11 is fastest. | 9 |
| | Volume: | µL/tube dispensed range:<br>▪ 96-tube manifold: 50-3000<br>▪ 192-tube manifold: 25-3000<br>▪ 1536-well (32-tube dispenser): 03-3000 | µL/tube<br>96 = 50<br>192 = 25<br>32 = 10 |
| | Number: | For 1536-well- 32-tube dispenser: the number of pre-dispenses to perform. | 2 |
| | **Bottom wash** | Bottom washing adds an initial wash cycle to the specified number of cycles. Fluid is simultaneously dispensed and aspirated to create cleaning turbulence (at the specified height). The manifold descends to aspirate again and ends with a final dispense to fill the wells. Not available for 1536-well plates. | |
| | Rate: | Valid range is 3-11. The cell wash rates, 1 CW and 2 CW, which use low-flow tubing, are available but not recommended. Cell wash options are designed for gentle washing, while bottom wash is designed for vigorous washing. | |
| | Wash Volume: | 25-3000 µL/well dispense | 250 |
| | Positioning: | X- and Y- horizontal axes, Z- height (vertical) axis can be adjusted. Repositioning the tubes to harder-to-reach areas of the wells may the improve results. | |

| Keypad name | Option | Description / Values range | | | | Default values |
|---|---|---|---|---|---|---|
| MIDCYC | Between cycles | | | | | |
| | **Shake** | To mix the contents of the plate. | | | | No |
| | Duration | From 1 second to one hour. | | | | 5 sec. |
| | Intensity | Slow | Medium | Fast | Variable | Med |
| | | 3.5 Hz | 5 Hz | 8 Hz | | |
| | **Soak** | Delays wash for the duration to allow fluids in the plate to steep or bathe. | | | | No |
| | Duration | From 1 second to one hour. | | | | 30 |
| | **Home carrier** | To perform the shake or soak in the home position or not. However, the plate carrier is moved home when the total time of the shake and soak durations exceeds 1 minute. The vacuum pump is turned off in this scenario, so this action prevents drops from the manifold contaminating the plate. | | | | No |
| | **Pre-dispense between cycles** | To wet or condition the manifold tubes between cycles, which is only needed after a long soak. Same parameters as regular pre-dispense. | | | | No |
| POST | Post wash | When all cycles are completed. | | | | |
| | **Final Aspirate** | A final aspiration is performed to completely evacuate the wells. Same parameters as regular aspirate step. | | | | Yes |
| FORM | Plate format | Manner of processing large-format plates | | | | Plate |
| | Sector | Performs the entire wash step on one sector of the plate before it moves to the next sector. | | | | |
| | Plate | Performs each cycle to the entire plate before it starts the next cycle. | | | | |

**→>DEFINE>ADD** or **EDIT>WASHR>PRIME**

| Keypad | Option | Description / Values range | Default values |
|---|---|---|---|
| PRIME | **Prime** | Flush tubing to remove air bubbles. | |
| | **Prime Rate:** | Same as washer flow rate. | 9 |
| | **Volume:** | 5-999 mL | 40 mL |
| | **Low-Flow Volume:** | Some fluid must be primed through low-flow tubing to prevent air bubbles from forming. | 5 mL |
| | **Submerge Tips:** | To soak the tubes in the priming fluid for a specified duration for cleaning or maintenance purposes. If yes, set duration, up to 24 hours, in minutes. | No |

→>**DEFINE**>**ADD** or **EDIT**>**WASHR**>**AUTOCLEAN**

| Keypad | Option | Description / Values range | Default values |
|---|---|---|---|
| ACLEAN | **AutoClean** | Fills the priming trough with fluid, submerges the tips and runs the ultrasonic cleaner for the specified duration. | |
| | **Buffer:** | Buffer bottle selection, if applicable. | A |
| | **Duration:** | 0-24 hours in minutes. | 60 min. |

## Peri-pump Step Parameters

→>**DEFINE**>**ADD** or **EDIT**>**PERIP**>

❖ Priming and purging the cassette can also be accomplished using the Setup Menu>Peri: press and hold the PRIME key to flush fluid through the cassette or hold the PURGE key to reverse the fluid direction.

| Keypad name | Option | Description / Values range | Default values |
|---|---|---|---|
| PRIME | **Volume:** | 1-3000 µL To remove air from the tubing. | 300 |
| DISP | **Dispense** | The per-well volume to dispense.<br>▪ 1 µL cassette = 1 - 50 µL<br>▪ 5 µL cassette = 5 - 2500 µL<br>▪ 10 µL cassette = 10 - 3000 µL | 10 |
| | **Flow rate:** | The rate, µL/second/tube, that fluid is dispensed for each cassette type:<br>Cassette Type / 1 µL / 5 µL / 10 µL<br>(µL/sec/tube)<br>Low: 56 / 120 / 140<br>Medium: 60 / 140 / 160<br>High: 64 / 160 / 180 | HIGH |
| | **Cassette type:** | To require a specific cassette type for this protocol. If yes, select the type, if no, select ANY. | ANY |
| | **Plate type:** | Select the plate type, and optionally, limit the columns dispensed to. | 96 |
| | **Positioning:** | X- and Y- horizontal axes, Z- height (vertical) axis can be adjusted to improve performance. Default Z-axis for 1536-well plates is 254 steps. (11.61 mm) | Z = 336 steps (15.362 mm) |
| | **Pre-dispense:** | When enabled, dispenses into the priming trough immediately before filling the plate. Pre-dispense is recommended for most applications. It normalizes the tips to ensure precise fluid distribution. Set volume and number of pre-dispenses. | 10 µL<br>2 cycles |

| Keypad name | Option | Description / Values range | Default values |
|---|---|---|---|
| PURGE | **Volume:** | 1-3000 µL To preserve fluid in the tubing by pumping it back into the supply vessel, i.e. reverses the flow direction. | 300 |

## Syringe Dispenser Step Parameters

→>**DEFINE**>**ADD** or **EDIT**>**SYRNG**>

| Keypad name | Option | Description / Values range | Default values |
|---|---|---|---|
| PRIME | **Flow rate:** | 1-5 (See dispense step description) | 5 |
| | **Volume:** | 80-8000 µL. To remove air from the tubing. | 5000 |
| | **Syringe:** | A or B | A |
| | **Cycles:** | Number of prime cycles to perform | 2 |
| | **Pump delay:** | 0-5000 msec. When dispensing highly viscous fluids, the tubing's check valves perform more slowly, i.e. it takes longer to open and close them. Delaying the syringe pump sufficiently to allow the specified amount of fluid to pass through the check valves before being pumped into the syringe has been shown to improve dispense accuracy. Begin by setting the delay to 500 msec. Experiment with different settings to determine the optimal value for your fluid. | 0 |
| | **Submerge tips:** | To soak dispense tubes in the priming fluid for a specified duration for cleaning or maintenance purposes. If yes, set duration, up to 24 hours, in minutes. | 0 |
| DISP | **Flow rate:** | Rates 1-5 are dependent on the volume and plate type, except for 1536-well plates. See below. | 2 |
| | **Dispense Volume:** | 5-3000 µL depending on the plate type. | 10 |
| | **Syringe:** | A or B, or Both, when processing 1536-well plates. | A |
| | **Columns:** | Select the plate type, and optionally, limit the columns dispensed to. | 96 |
| | **Pump delay:** | (Same as Prime step description above.) | |
| | **Positioning:** | X- and Y- horizontal axes, Z- height (vertical) axis can be adjusted to improve performance. Default Z-axis for 1536-well plates is 254 steps. (11.61 mm) | Z = 336 steps (15.362 mm) |

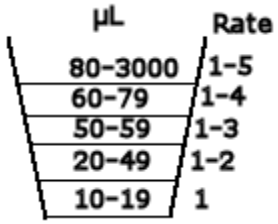
Be sure to use the **prime trough inserts** to submerge the tips in fluid.

| Keypad name | Option | Description / Values range | Default values |
|---|---|---|---|
| | **Pre-dispense:** | When enabled, dispenses into the priming trough immediately before filling the plate. Pre-dispense is recommended for most applications. It normalizes the tips to ensure precise fluid distribution. Set volume and number of pre-dispenses. | 10 µL<br>2 cycles |

## Syringe dispenser flow rates:

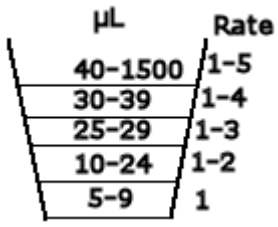
**Rates are volume and plate-type dependent:** For example, rate 1 must be used when dispensing between 10-19 µL to a 96-well plate. When dispensing 20-49 µL to a 96-well plate, you can use rates 1 or 2. And, when dispensing 50-59 µL to a 96-well plate, you can use rates 1, 2, or 3. And so on, as shown in the tables above.

**96-well plate:**




| Volume (µL) | Rate | µL/sec/well |
|---|---|---|
| 10-19 | 1 | 450 |
| 20-49 | 1-2 | 600 |
| 50-59 | 1-3 | 750 |
| 60-79 | 1-4 | 900 |
| 80-3000 | 1-5 | 1000 |

**384-well plate:**




| Volume (µL) | Rate | µL/sec/well |
|---|---|---|
| 5 -9 | 1 | 225 |
| 10-24 | 1-2 | 300 |
| 25-29 | 1-3 | 375 |
| 30-39 | 1-4 | 450 |
| 40-1500 | 1-5 | 500 |

❖ Note: the **µL/sec/well** rate accounts for 2 tubes/well when addressing 96-well plates and one tube/well for 384-well plates.

**1536-well plate:**

| Flow rates for the 32-tube dispense manifold do not have minimum volumes. | | |
|---|---|---|
| The default rate is 2. | Volume (µL) | Rate |
| | 3-3000 | 1-5 |

## Shake Step Parameters

<table>
<tr><th>Keypad name</th><th>Option</th><th>Description / Values range</th><th>Default values</th></tr>
<tr><td>SHAKE</td><td>Yes or No</td><td>Yes to shake the plate for a defined period.</td><td>No</td></tr>
<tr><td rowspan="3"></td><td>Duration:</td><td>Up to 1 hour in seconds.</td><td>5</td></tr>
<tr><td>Intensity:</td><td>Slow, medium, fast or variable, which cycles through the other levels of intensity.<br><table><tr><th>Intensity</th><th>Herz</th><th>RPM</th></tr><tr><td>Slow</td><td>3.5</td><td>210</td></tr><tr><td>Medium</td><td>5</td><td>300</td></tr><tr><td>Fast</td><td>8</td><td>480</td></tr></table></td><td>Medium</td></tr>
<tr><td>Soak:</td><td>Yes or No. To allow buffer/dispense fluid to remain (steep) in wells for the specified duration. If yes, set the duration, up to one hour. 30 second default duration.</td><td>No</td></tr>
<tr><td></td><td>Move carrier to home:</td><td>Regardless of user selection, the plate carrier is moved home when the total time of the shake and soak durations exceeds 1 minute. The vacuum pump is turned off in this scenario ; moving the plate home prevents drops from the manifold contaminating the plate.</td><td>Yes</td></tr>
</table>

# Pre-defined Protocols

The Maintenance and QC protocols are referenced in the Recommended Maintenance and Instrument Verification Schedules. The "Sample" protocols are provided to facilitate learning. When using the **LHC software**, you may need to customize these protocols to match your instrument's settings.

- No Buffer Switching module? **Copy** the protocol and modify it for your instrument. See page 69.
- Please read the important information about running maintenance protocols when the BioStack is under keypad control in the **Maintenance** chapter.
- **Copy the protocol** to modify it or to create a duplicate. When using:
  - LHC: select **File>Save As**,
  - Keypad: → >**DEFINE**>**COPY**

  and assign a unique name to the copy.

Keypad protocol number

**Maintenance Protocols**

| **Daily Maintenance** | | **#** |
|---|---|---|
| W-DAY_RINSE | Simple one-step protocol to fully flush the system with water or reagent to keep the manifold tubes clog-free. Defined for use with Buffer A; 500 mL total volume. | 01 |
| S-DAY_RINSE_A&B | Two-step protocol to flush tubing for both syringe pumps (A & B). 16000 µL total volume. | 02 |
| P-1UL_CASS_RINSE | Simple one-step protocol to flush Peri-pump tubing for 1 µL cassette. | 03 |
| P-5UL_CASS_RINSE | Simple one-step protocol to flush Peri-pump tubing for 5 µL cassette. | 04 |
| P-10UL_CASS_RNSE | Simple one-step protocol to flush Peri-pump tubing for 10 µL cassette. | 05 |
| W-OVERNIGHT_LOOP (for LHC users only*) | Protocol designed to keep the manifold in a wetted condition overnight or for a long downtime period; manifold tubes are submerged in fluid for 4-hour intervals between primes in this virtually endless loop. Defined to use Buffer A. | n/a |
| *Keypad-Stand alone operation | If you are not using the LHC software to control your EL406, use the AutoPrime feature or the alternative procedure described in the Maintenance chapter as a substitute for the Overnight_Loop protocol. | |
| W-RINSE_AND_SOAK | Identical to W-DAY_RINSE with one addition, the manifold tubes are submerged and soaked for 5 minutes in the fluid. | 06 |
| W-Decontaminate W-DECON (onboard) | Aids implementation of the recommended decontamination routine. This protocol expects to first | 07 |

| | | |
|---|---|---|
| | flush the system with disinfectant from Buffer A and later rinse with water using Buffer B. | |
| S-Decontaminate S-DECON (onboard) | This protocol requires first running disinfectant and later running water through the system; for Syringe A only. It can be easily modified to suit any major cleaning effort. | 08 |
| W-LONG_SHUTDOWN | Helps implement the routine recommended for preparing the instrument for storage. This protocol expects to run disinfectant from Buffer A, then water from Buffer B, and lastly, air through the system - remove bottle from Buffer C valve. | 09 |
| S-LONG_SHUTDOWN | Prepares the instrument for long-term storage. This protocol requires first running disinfectant, then running water through the system, and lastly, air through the system. Defined to use Syringe A. | 10 |
| S-DAY_RINSE_A | Identical to S-DAY_RINSE_A&B except only Syringe A is defined. | 11 |
| W-CLEAN_w-BUFFER | Combines priming and AutoClean steps to clean and rinse the wash manifold. It is defined to obtain cleaning fluid from Buffer B, and rinse fluid from Buffer A. The protocol must be modified to run on instruments without Buffer Switching. | 12 |
| W-PRIME_200 | Simple prime routine; defined for Buffer valve A only. | 13 |
| W-PRIME_ALL_BFRS | Consecutively primes each of the Buffer Switching valves beginning with D. | 14 |

| **QC (Quality Control) Protocols** | | # |
|---|---|---|
| W-96_DISP_TEST | Dispense precision test protocol for 96-tube manifold. | 15 |
| W-96_EVAC_TEST | Evacuation efficiency test protocol for 96-tube manifold. | 16 |
| W-192_DISP_TEST | Dispense precision test protocol for 192-tube manifold. | 17 |
| W-192_EVAC_TEST | Evacuation efficiency test protocol for 192-tube manifold. | 18 |
| W-1536_EVAC_TEST | Evacuation efficiency test protocol for 128-tube manifold. | 19 |
| SA-1536_DISP_TST | Dispense precision test protocol for 32-tube Syringe A manifold. | 20 |
| SB-1536_DISP_TST | Dispense precision test protocol for 32-tube Syringe B manifold. | 21 |
| P-1536_DISP_TEST | Dispense precision test protocol for Peri-pump 1 µL cassette and 1536-well plate. | 22 |

**Sample Protocols (LHC Only)**

| Serial dilutions | Description |
|---|---|
| P-96_DILUTION | Peri-pump serial dilution protocol dispenses 20 µL to 240 µL in 20 µL increments to each column of the plate. 20 µL to column 1; 40 µL to column 2; 60 µL to column 3; and so on till 240 µL to column 12. |
| P-384_DILUTION | Serial dilution protocol dispenses 2 µL to 94 µL in 2 µL increments to each column of the plate. 2 µL to column 1; 4 µL to column 2; and so on till 94 µL to column 24. |
| S-96_DILUTION | Syringe dispenser serial dilution protocol dispenses 240 µL to 20 µL in 20 µL increments to each column of the plate. 240 µL to column 1; 220 µL to column 2; 200 µL to column 3; and so on till 20 µL to column 12. Defined for Syringe A. |
| S-384_DILUTION | Serial dilution protocol dispenses 98 µL to 6 µL in 4 µL increments to each column of the plate. 98 µL to column 1; 94 µL to column 2; 90 µL to column 3; and so on till 6 µL to column 24. Defined for Syringe A. |
| **Cell Wash** | |
| W&P-96_CELL_WASH | Cell wash-dispense protocol uses the special low-flow tubing, optimal dispense and aspirate heights, and vacuum delay during the wash step. Following the wash, the Peri-pump dispenses 200 µL to each well. |
| **Microplate Manufacturers** | |
| W-COSTAR_FLAT | Standard wash protocols modified to best position the manifold tubes for dispensing and aspirating to Costar® flat-bottomed and round-bottomed wells. |
| W-COSTAR_ROUND | |
| W-NUNC_384 | Standard wash protocols modified to best position the manifold tubes for dispensing and aspirating to Nunc® flat-bottomed wells, round-bottomed wells, and 384-well plates. |
| W-NUNC_FLAT | |
| W-NUNC_ROUND | |

Chapter 4

# Maintenance

This chapter describes how to maintain the EL406 Washer Dispenser, prepare it for storage or shipment, and decontaminate the instrument.

# Overview

A **Preventive Maintenance (PM)** regimen for the EL406 includes rinsing and soaking the fluid path and cleaning and/or autoclaving the various components. The level of maintenance required to keep the instrument performing as expected is dependent on several factors, including the type of fluid dispensed, the frequency of use, and the work habits employed.

The chart on the next page summarizes BioTek's recommended maintenance tasks, and indicates approximately how often each task should be performed. Daily and periodic routines and minimal guidelines for frequency are listed. Beyond that, it is difficult for BioTek to recommend a fixed frequency for each task to be performed. The frequency of conducting these tasks must be based on the risk and performance factors of your assays.

Develop a maintenance schedule for your EL406 based on the characteristics of the fluids used and the activity level. Here are some guidelines for each component:

### Washer

- When using fluids prone to dry and harden quickly, the washer's dispense and aspirate tubes can clog quickly, and must be rinsed frequently and cleaned regularly. Run **AutoClean** ultrasonic cleaning regularly.
- If the washer will be idle for several hours or days at a time, the tubes should be soaked to keep them in a "wetted" state. Enable the **AutoPrime** program if the washer is idle for more than 3 hours.
- Wash solutions affect the rinse frequency. If the solution does **not** contain surfactant, consider rinsing (or running AutoPrime) at least once an hour.

### Peri-pump

- Purge the fluid at the end of a dispense run and flush the tubing with water (or buffered saline and then water). This is a good practice whenever the dispenser will be idle for more than an hour, and at the end of the day.
- When using the 1 µL cassette, filter fluids to 50 microns to reduce the chance of the tips clogging.
- When dispensing fluids that can crystallize or harden after use, increase the frequency of maintenance activities. Autoclave the cassette as needed.
- Keep track of the number of plates processed with a cassette to determine when the cassette has reached its expected lifetime and is due for replacement or recalibration.

### Syringe Dispenser

- Perform the daily maintenance routines. Flush the dispenser with an appropriate reagent at the beginning of the day (e.g., deionized water in the morning) and at the end of a run.
- When dispensing fluids that can crystallize or harden after use, increase the frequency of maintenance activities. This is especially important for the 32-tube small bore (SB) dispensers.
- Autoclave the Syringe heads and pistons, and the 16-tube manifolds as needed.

❖ **Important**: Do **NOT** autoclave the 32-tube Syringe dispense manifolds.

- Enable **AutoPrime** with a soaking period for overnight, especially for the 32-tube SB models, and perform the special cleaning procedures as needed to keep the tubes clog-free.

## Schedule

The following chart recommends preventive maintenance tasks, the frequency with which each task should be performed, and the pre-defined onboard Maintenance program that should be run (if applicable).

❖ It is important to note that the risk and performance factors associated with your assays may require that some or all of the procedures be performed more frequently than presented in this schedule.

# Recommended Maintenance Schedule

| Tasks | Frequency | | | | |
|---|---|---|---|---|---|
| | Daily | Overnight / Multi-Day | Weekly | Periodic / Monthly | Before storage / shipment |
| **Washer** | | | | | |
| Run W-DAY_RINSE | ✓ | ✓ | | | |
| Run AutoPrime | ✓ | ✓ | | | |
| Run W-OVERNIGHT_LOOP (LHC only) | | ✓ | | | |
| Run W-RINSE_AND_SOAK | | ✓ | | | |
| Run AutoClean | | | | ✓ | ✓ |
| **Peri-pump Dispenser** | | | | | |
| Flush dispense cassette | ✓ | ✓ | | | |
| Record approximate number of plates processed with cassette | ✓ | | | | |
| **Syringe Dispenser** | | | | | |
| Run S-DAY_RINSE_A&B | ✓ | ✓ | | | |
| Run AutoPrime | ✓ | ✓ | | | |
| **Clean Components** | | | | | |
| Remove protein residuals and fungi growth, (if necessary) | ✓ | | ✓ | ✓ | |
| Check/empty waste bottles | ✓ | | | | ✓ |
| Clean bottles | | | | ✓ | ✓ |
| Clean plate carrier system and transport arm | | | ✓ | | ✓ |
| Clean washer manifold | | | | ✓ | ✓ |
| Clean aspirate and dispense tubes | | | | ✓ | ✓ |
| Clean exterior surfaces and mist shield | | | ✓ | | ✓ |
| Clean fluid inlet filter | | | | ✓ | ✓ |
| Clean Syringe dispenser manifold | | | | ✓ | ✓ |
| Clean Syringe dispenser tubes | | | | ✓ | ✓ |
| Autoclave Syringe pumps | | | | ✓ | |
| **Decontaminate** | | | | | |
| Decontaminate external surfaces | | | | ✓ | ✓ |
| Run W-DECONTAMINATE (LHC)<br>Run W-DECON (Keypad) | | | | ✓ | ✓ |

| | | | | | |
|---|---|---|---|---|---|
| Run S-DECONTAMINATE (LHC)<br>Run S-DECON (Keypad) | | | | ✓ | ✓ |
| **Prepare for Storage or Shipment** | | | | | |
| Run W-LONG_SHUTDOWN | | | | | ✓ |
| Run S-LONG_SHUTDOWN | | | | | ✓ |
| **Replace / Repair Components** | | | | | |
| Replace washer manifold o-rings and channel-end seals | *Annually* | | | | |
| Recalibrate Peri-pump cassette | *As Needed* | | | | |
| Replace Peri-pump dispense tips | *As Needed* | | | | |
| Replace Peri-pump cassette tubing | *As Needed* | | | | |
| Replace Syringe manifold check valves and plugs | *As Needed* | | | | |

# Required Materials

For rinsing/soaking the fluid path, and for cleaning the components:

- Deionized or distilled water
- Buffered saline solution or enzyme-active detergent for protein or cell-based assays
- Sodium hypochlorite (NaClO or bleach)
- 70% isopropyl alcohol (or ethanol)
- Dispense and aspirate tube styluses (supplied with the instrument)
- Phillips head screwdriver
- 9/64" (3.57 mm) hex wrench (supplied with the instrument)
- 3/32" (2.39 mm) hex wrench (supplied with the instrument)
- Lint-free disposable towels
- Dish soap or other mild cleaner
- Soft-bristled brush
- Protective gloves, biohazard trash bags, lab coat, safety glasses, surgical mask

For replacing washer manifold o-rings and channel-end seals:

- O-rings (PN 49941)
- Channel-end seals (PN 49486)

❖ Not applicable to the 128-tube aspirate manifold.

For replacing Syringe dispenser manifold plugs and check valves:

- Manifold plugs (PN 45090)
- Autoclavable check valves for use with non-organic substances (PN 68083) or check valves recommended for use with organic substances (PN 68073), which cannot be autoclaved.

❖ In the cleaning procedures, when not otherwise specified, "water" means either deionized or distilled water.

❖ Unless otherwise instructed, **always** connect a supply bottle containing deionized or distilled water to the washer before running the Maintenance programs.

# Warnings & Precautions

Please read the following before performing any Maintenance procedures:

| | |
|---|---|
|  | **Warning! Internal Voltage.** Turn off and unplug the instrument for all maintenance and repair operations. |
|  | **Caution! Chemical Compatibility with Washers.** Some chemicals may cause irreparable damage to washers. The following chemicals have been deemed safe for use in washers: buffer solutions (such as PBS), saline, surfactants, deionized water, 70% ethyl, isopropyl, or methyl alcohol, 40% formaldehyde, and 20% sodium hydroxide.<br><br>Never use DMSO or other organic solvents. These chemicals may cause severe damage to the instrument. See ***Appendix C, Chemical Compatibility,*** for more information. Use of wash buffers containing acetic acid is limited to washers upgraded with BioTek part number 68098 Teflon® valves.<br><br>Contact BioTek for upgrade information and prior to using other questionable chemicals. |
|  | **Warning!** Wear protective gloves when handling contaminated instruments. Gloved hands should be considered contaminated at all times; keep gloved hands away from eyes, mouth, nose, and ears. |
|  | **Warning!** Mucous membranes are considered prime entry routes for infectious agents. Wear eye protection and a surgical mask when there is a possibility of aerosol contamination. Intact skin is generally considered an effective barrier against infectious organisms; however, small abrasions and cuts may not always be visible. Wear protective gloves when handling contaminated instruments. |
|  | **Important!** Do not immerse the instrument, spray it with liquid, or use a “wet” cloth on it. Do not allow water or other cleaning solution to run into the interior of the instrument. If this happens, contact BioTek’s Technical Assistance Center. |

# Maintenance Protocols

BioTek provides the following pre-defined **Maintenance protocols**:

| Daily Maintenance | | |
|---|---|---|
| W-DAY_RINSE | Simple one-step protocol to fully flush the system with water or reagent to keep the manifold tubes clog-free. Defined for use with Buffer A; 500 mL total volume. | 01 |
| S-DAY_RINSE_A&B | Two-step protocol to flush tubing for both syringe pumps (A & B). This protocol fully primes each Syringe system, with 40 mL total volume per pump. | 02 |
| P-1UL_CASS_RINSE | Simple one-step protocol to flush Peri-pump tubing for 1 µL cassette. | 03 |
| P-5UL_CASS_RINSE | Simple one-step protocol to flush Peri-pump tubing for 5 µL cassette. | 04 |
| P-10UL_CASS_RNSE | Simple one-step protocol to flush Peri-pump tubing for 10 µL cassette. | 05 |
| W-OVERNIGHT_LOOP (LHC only*) | Protocol designed to keep the manifold in a wetted condition overnight or for a long downtime period; manifold tubes are submerged in fluid for 4-hour intervals between primes in this virtually endless loop. Defined to use Buffer A. | N/A |
| | * Keypad users can use AutoPrime as an alternative. | |
| W-RINSE_AND_SOAK | Identical to W-DAY_RINSE with one addition, the manifold tubes are submerged and soaked for 5 minutes in the fluid. | 06 |

| Periodic Maintenance | | |
|---|---|---|
| W-DECONTAMINATE W-DECON (onboard) | Aids implementation of the recommended decontamination routine. This protocol expects to first flush the system with disinfectant from Buffer A and later rinse with water using Buffer B. | 07 |
| S-DECONTAMINATE S-DECON (onboard) | This protocol requires first running disinfectant and later running water through the system; for Syringe A only. It can be easily modified to suit any major cleaning effort. | 08 |
| W-LONG_SHUTDOWN | Helps implement the routine recommended for preparing the instrument for storage. This protocol expects to run disinfectant from Buffer A, then water from Buffer B, and lastly, air through the system - remove bottle from Buffer C valve. | 09 |
| S-LONG_SHUTDOWN | Prepares the instrument for long-term storage. This protocol requires first running disinfectant, then running water through the system, and lastly, air through the system. Defined to use Syringe A. | 10 |
| S-DAY_RINSE_A | Identical to S-DAY_RINSE_A&B except only Syringe A is defined. | 11 |
| W-CLEAN_w-BUFFER | Combines priming and AutoClean steps to clean and | 12 |

| Periodic Maintenance | | |
|---|---|---|
| | rinse the wash manifold. It is defined to obtain cleaning fluid from Buffer B, and rinse fluid from Buffer A. The protocol must be modified to run on instruments without Buffer Switching. | |
| W-PRIME_200 | Simple prime routine; defined for Buffer valve A only. | 13 |
| W-PRIME_ALL_BFRS | Consecutively primes each of the Buffer Switching valves beginning with D. | 14 |

**To Run a Maintenance Protocol:**

❖ LHC users: follow the recommendation on page 108 to customize the protocol for **First-time Use**.

BioTek provides numerous pre-defined protocols for maintaining the instrument in top condition. Protocols are stored onboard the instrument and installed on your PC by the EL406 Interface Software (IS) installation wizard. The files are stored in the LHC's default file location. Review the LHC's Help for more details.

| Using the | Description |
|---|---|
| **Keypad** | 1. At the main menu, press **RUN**.<br>2. Press **Options** to scroll to the desired protocol or use the arrow and number keys to enter its number.<br>3. Press ENTER and follow the prompts. |
| **LHC** | 1.  Select **Open** and locate the EL406 folder.<br>2. Open the EL406 folder to access the **Maintenance Protocols** folder.<br>3. Select the desired protocol. |

❖ To halt a maintenance program in progress, press the **STOP** key.

**Important info for those using the BioStack under keypad control:**

The EL406 does not distinguish between protocols designed to process plates and maintenance protocols. When the instrument is configured to use the BioStack, every protocol tells it to deliver plates for processing, and the protocol is run repeatedly until all the plates in the input stack have been processed.

When you have the BioStack under keypad control (rather than LHC control), to perform the required maintenance protocols and/or to prime the system with fluid prior to processing plates, you must choose one of these methods to perform maintenance activities:

**Maintenance options with the BioStack configured for keypad control:**

| 1 | 2 |
|---|---|
| **Quick Wash / Quick Dispense:** Use the PRIME option of the Quick Wash and Quick Dispense menus instead of running the pre-defined protocol.<br>• **Quick Wash**: set the prime volume to 500 mL.<br>• **Quick Dispense**: for each dispenser, PRIME the tubing till all visible air bubbles are removed.<br>Use this option to prime the tubing just before processing plates and whenever it is most convenient. | **Disengage the BioStack**: Change the BioStack operating mode on the instrument to run the pre-defined protocols:<br>1. Press **Setup Menu**.<br>2. Select →<br>3. Select **BIOSTK**.<br>4. Select **CONF**.<br>5. Select **Manual**.<br>Restore the BioStack mode when you want to process plates. |

### LHC First-time Use:

When using the LHC, you may have to modify the pre-defined protocols to meet your assay requirements and to match your instrument configuration. In addition to action steps, every protocol file contains **Instrument Settings**, including COM port, manifold type, and so on. Edit the protocol to match your instrument's COM Port and other configuration details.

With the EL406 connected to and communicating with the host computer:

1.  Click **Open**, locate the **EL406** folder and click **Open**.
2. Open the **Maintenance Protocols** folder and select the desired protocol.
3.  Change the COM port if necessary: click the **Port** link and enter the correct value.
4.  Click the **Settings** link, which opens the Instrument Settings dialog.
5. Under Get settings from: click the **instrument** link.
6.  Save the protocol.

# Rinse/Soak the Fluid Path

## Daily Maintenance

Daily maintenance involves flushing the washer and dispensers with an appropriate reagent or deionized water throughout the day. Routine rinsing helps to prevent the aspirate and dispense tubes from clogging between runs.

The recommended **rinsing frequency** depends on the solutions currently in use:

- If a solution containing surfactant is used throughout the day, the rinsing procedure should be performed if the device is idle for more than 3 hours.
- If the solution does not contain surfactant, consider rinsing at least once an hour.

❖ The type of hardware also affects rinsing frequency. The 32-tube dispense manifolds require more diligence to keep them clog-free.

Run these protocols and enable **AutoPrime** to satisfy daily maintenance requirements:

- W-DAY_RINSE
- S-DAY_RINSE_A&B
- P-#UL_CASS_RINSE (# represents the cassette type)

Make sure the supply bottles contain sufficient rinse solution and that the waste bottles are empty before running the protocols.

See the additional maintenance procedures on page 112 when dispensing protein solutions.

### AutoPrime

Enable **AutoPrime** to automatically wet or soak the washer's and Syringe dispenser's tubes after a user-specified amount of idle time. Learn about AutoPrime, including how to enable it in ***Chapter 3, Operation***.

❖ Press the **STOP** button on the keypad to interrupt the AutoPrime routine when it is underway.

❖ Any interaction with the instrument via the keypad or the LHC resets the interval clock.

❖ The instrument must be at its main menu to run AutoPrime.

## Overnight/Multi-Day Maintenance

Overnight/multi-day maintenance involves flushing all solutions out of the instrument, and then periodically rinsing and soaking the tubes to keep them moist. Here are three recommendations for accomplishing the task. Employ the method that best suits your work flow:

- **AutoPrime**
- **Overnight Loop** (LHC only) for wash manifold
- **Submerge and shutdown**

### Maintaining 1536-well Hardware

At the end of the day run **W-DAY_RINSE** to flush the 128-tube aspirate manifold. The aspirate tubes cannot completely empty the priming trough when the run is finished. Remove the fluid manually with a pipette or paper towel.

The 32-tube-SB dispense manifolds can become easily clogged. It is especially important to enable **AutoPrime** with the suggested soaking periods (next page) to ensure trouble free startup at the beginning of the next day.

### AutoPrime

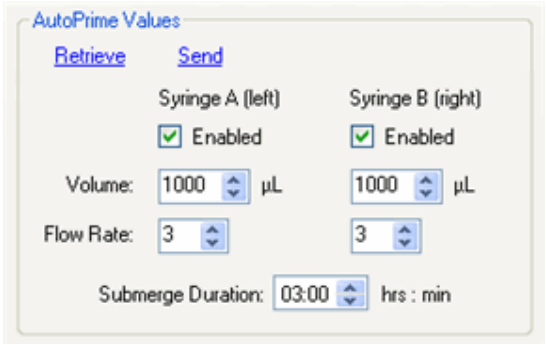
AutoPrime can be used to keep the manifold tubes wetted during idle periods throughout the day and then modified to soak the tubing for longer periods overnight and on the weekends. Learn about **AutoPrime**, including how to enable it in ***Chapter 3, Operation***.

**Washer AutoPrime Parameters**

| |
|---|
| AutoPrime Interval = 15 minutes |
| Device = Washer |
| AutoPrime = Yes |
| Volume = 60 µL/tube |
| Rate = 09 |
| Buffer = Any |
| Duration: 120 minutes |

To repeatedly soak both devices, make sure the AutoPrime interval is less than the total combined submerge duration.

**Syringe AutoPrime Parameters**




For overnight maintenance, define the AutoPrime values to fill the priming-trough-inserts and then submerge the tubes for several hours.

When the EL406 is set up for 1536-well washing, you can disable AutoPrime for the washer; typically the 128-tube aspirate manifold does not require the same amount of soaking as dispense manifolds.

### 32-tube Syringe Dispenser

When using the 32-tube dispense manifolds, especially the small bore (SB) model, use the following **AutoPrime** parameters to keep the manifold clog-free:

| | |
|---|---|
| Volume: | 1000 µL |
| Flow rate: | 3 |
| Submerge duration: | 3 hours (requires priming trough inserts) |

- Enable AutoPrime for both Syringe manifolds and disable AutoPrime for the Washer.

**Overnight / Multi-day practice:** After modifying the **AutoPrime** parameters to submerge the tubes for several hours:

1. Put the priming-trough-inserts into the priming trough.
2. Use **Quick Dispense** to fill both inserts (prime cups): **DISP>PRIME** (for both Syringe A and B). Run the **Prime** two or three times to fill the cups.
3. When the priming troughs are filled, press **Main Menu**.

## Overnight Loop

To keep the wash manifolds in a wetted condition, you can run these pre-defined protocols to soak the tubes for several hours at a time:

- W-OVERNIGHT_LOOP: requires the washer to remain turned on; available for LHC users only.
- W-RINSE_AND_SOAK: alternatively, run this protocol, number 06 on the keypad, and turn off the instrument after the soak begins. The tubes will soak in the priming trough until the instrument is turned on again.

## Submerge Tips and Shutdown for Overnight Soaking

You can simultaneously soak the EL406 dispense manifolds by filling the priming troughs and turning off the instrument after the soak begins. The tubes will soak in the priming troughs until the instrument is turned on again.

First run S-DAY_RINSE_A&B but do not empty the priming trough inserts, then run W-RINSE_AND_SOAK. When the manifold is submerged, turn off the instrument. The syringe dispenser manifolds will lower into their troughs.

## Removing Protein Residuals

**Important!** Solutions containing proteins, such as bovine serum albumin (BSA), will compromise the EL406's performance over time unless a strict maintenance regime is adhered to.
**Do not use alcohol to flush out BSA.**

When dispensing protein solutions or similar fluids with the washer and the Syringe dispenser, perform these additional procedures to thoroughly flush out protein particles and other contaminants from the fluid path.

- **Four-liter** volumes specified in the following are approximate amounts.
- **S-DECONTAMINATE** (S-DECON): this pre-defined protocol specifies Syringe A. Make a copy of the protocol to execute for Syringe B.

### Daily Practice with buffer or deionized water:

If the washer or dispenser will be idle between plates for longer than 45 minutes, flush the proteins from the washer:

1. Fill a supply bottle with deionized water. Connect the bottle to the washer or dispenser. (Buffer valve "A" if the Buffer Switching module is connected.)
2. Run the applicable **DAY_RINSE** protocol.
3. Enable **AutoPrime** for 60-minute intervals.

At the end of the day:

1. Fill a supply bottle with deionized water. Connect the bottle to the washer dispenser (Buffer valve "A" if the Buffer Switching module is connected.)
2. Run the applicable **DAY_RINSE** protocol three times.
3. Perform your regular Overnight/Multi-Day Maintenance routine.

### Weekly or As Needed use NaOH and HCl to remove proteins:

1. Flush the system with 0.1-0.5 N* NaOH (sodium hydroxide), followed by neutralization with an equivalent normality (0.1-0.5 N) of HCl (hydrochloride).
2. Rinse well with deionized water to remove the HCl.
3. Run the applicable **DAY_RINSE** protocol three times with deionized water if you plan to use the device immediately.

- * N = Normal solution, which contains 1 'gram equivalent weight' (gEW) of solute per liter of solution. The gram equivalent weight is equal to the molecular weight expressed as grams divided by the 'valency' of the solute.

### Alternatively use an Enzyme-Active Detergent:

1. Mix an enzyme-active detergent according to the manufacturer's directions to fill a four-liter supply bottle. Connect the bottle to the washer's Buffer valve **A** or one of the Syringes. Connect a bottle of DI water to Buffer valve B to rinse the tubing.
2. Run the **W-DECONTAMINATE** (W-DECON (07)) or **S-DECONTAMINATE** (S-DECON (08)) protocol, as appropriate.
3. **LHC users**: Respond to the **Delay** messages, "Connect a bottle of water...", leave the detergent bottle connected and click **Continue**.
   For the **Syringes**: connect a bottle with DI water to the pump and **REPEAT** the protocol.
4. When the protocol is completed, connect a bottle containing four liters of deionized water and run **W-DAY_RINSE** or **S-DAY_RINSE** three times to flush the system.

# Periodic Maintenance

Periodic maintenance involves cleaning the components on a regular basis to keep the instrument running efficiently and in compliance with performance specifications. The recommended **frequency for cleaning components** is *at least monthly*. The risk and performance factors associated with your assays may require that some or all of the procedures be performed more frequently.

| | |
|---|---|
|  | **Warning! Internal Voltage.** Turn off and unplug the instrument for all cleaning operations. |
|  | **Important! Do not** apply lubricants to manifold o-rings, channel-end seals, bottle cover seals, any tubing connection, or any surface that is a part of the fluid path. The use of any lubricant on the fluid handling components will interfere with the aspirate and dispense performance, and may cause irreparable damage to these components. |
|  | **Important!** When cleaning components:<br><br>Do not immerse the instrument, spray it with liquid, or use a "wet" cloth on it.<br><br>Do not allow the cleaning solution to run into the interior of the instrument. (If this happens, contact the BioTek Service Department.)<br><br>Do not expose any part of the instrument to the recommended diluted sodium hypochlorite solution (bleach) for more than 20 minutes. Prolonged contact may damage the instrument surfaces. Be certain to rinse and thoroughly wipe all surfaces.<br><br>Do not soak the keypad. Instead, moisten a clean cloth with deionized or distilled water and wipe the keypad. Dry it immediately with a clean, dry cloth. |

### *Clean the Bottles*

- Clean and rinse the supply bottles with deionized water before the first use, before each refill, and, periodically, as necessary, to prevent bacteria growth.
- Empty the waste bottle often (at least daily), and firmly seat the waste bottle stopper.
- Rinse the covers every time the wash or rinse bottles are filled.

- Accumulated algae, fungi, or mold may require decontamination. Decontamination is described on page 131.

> ❖ To ensure that fluid does not back up into the vacuum pump during operation, always operate the instrument with the **waste sensor cable** installed and the **waste detection sensor** enabled (the sensor is enabled by default; see ***EL406 Settings and Utilities*** in ***Chapter 3***, ***Operation***).
> If fluid collects in the **overflow bottle**, thoroughly rinse the level-switch assembly and bottle.

- Check the hex nuts securing the quick-disconnects to the bottle cap to ensure they are not loose or corroded.

## *Clean the Plate Carrier*

If liquid has overflowed onto the plate carrier, transport rail, or glide strips, some buildup may occur and prevent the microplate from seating correctly on the carrier. This can interfere with plate transport. Weekly cleaning is recommended.

1. Turn the instrument off.
2. Lift the carrier up and off the transport rail.
3. Clean the carrier, rails, and glide strips, using mild detergent and hot water, 70% isopropyl alcohol, or ethanol. Clean the priming trough, too.
4. If detergent was used, wipe the components with a cloth moistened with water. Use a clean, dry cloth to dry the components.
5. To replace the carrier, place it on the transport rail so the slot on its base fits into the **Y-axis Carrier Arm**. If necessary, release the spring-loaded microplate clamp in the back left corner of the carrier to level the carrier on the base.

# Washer Maintenance

The EL406's washer manifold, mist shield, and tubing require periodic cleaning. Regular rinsing helps to keep the washer manifold clean, the aspiration and dispense tubes clear, and it increases the life of the tubing.

If you suspect a particular problem is related to the manifold (for example, clogged tubes can result in poor or uneven aspiration or dispensing), you should perform a thorough cleaning of the dispense and aspirate tubes and channels. When necessary, follow the decontamination procedure that begins on page 131 to disinfect the manifold and tubing.

## Clean the manifold, mist shield, and tubing

1. Run the system "dry":
   - Disconnect the fluid supply from the instrument,
   - Run **W-DAY_RINSE**
2. Turn off the instrument and disconnect the power cable.
3. Moisten a lint-free disposable towel with water, or with water and mild detergent. **Do not soak the cloth**.
4. Remove the mist shield if it is attached. Wipe the inside and outside surfaces of the mist shield with the towel. Wipe the top surface of the instrument base, and all exposed surfaces of the instrument.
5. If detergent was used, wipe all surfaces with a cloth moistened with water.
6. Use a clean, dry cloth to dry all wet surfaces.

## Remove and clean the washer manifold

- ❖ Hold the two manifolds together as a single unit when removing or replacing.

1 Using the 9/64" (3.57 mm) hex wrench, remove the screws, washers, and springs that hold the manifolds in place. Carefully remove the manifolds and end plates.

- ❖ Avoid pressing the stylus against the sides of the tubes during cleaning. This can cause the tubes to bend, which may negatively affect dispense precision.

2 Using a soft-bristled brush, thoroughly clean the outside of the manifolds. Clean the insides of each tube with the appropriate stylus (aspirate/dispense). Flush hot water through the cross channels.

3 Rinse the manifold with deionized or distilled water. Check to see if water comes out of all dispense and aspirate tubes. If not, soak the manifold in hot, soapy water and repeat.

**Caution.** When reinstalling the manifold, only tighten the screw-washer-spring assembly that holds it in place until you feel the mechanical stop. Tightening past this point will damage the instrument and will **void your warranty.**

4 When satisfied, reassemble the manifold and end plates, making sure that the two o-rings are in place prior to reassembly. **Do not overtighten the manifold screws.**

5 Install the mist shield.

6 Reconnect the power cable and turn on the instrument.

7 Prime the system with deionized water by running W-DAY_RINSE or a similar protocol. Watch for leaks. If fluid leaks out of the back of the instrument, firmly seat the tubing. If fluid leaks from the manifold, try disassembling and carefully reassembling.

8 Verify aspirate/dispense performance visually or by performing the tests in ***Chapter 5***, ***Qualification***.

- ❖ Replace the o-rings on an annual basis. Replace the channel-end seals (rubber plugs) if they show signs of cracking or drying out. See ***Washer O-Rings and Channel-End Seals*** on page 139.
- ❖ For additional cleaning, run AutoClean™ with Ultrasonic Advantage™.
- ❖ **DO NOT AUTOCLAVE** the manifold!

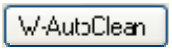
## *AutoClean™ the Washer*

| | |
|---|---|
|  | **Warning! Ultrasonic energy** is present in the cleaning reservoir when AutoClean is running. **Do not put your fingers in the bath!** Ultrasonic energy can be destructive to human tissue. |
|  | **Important!** Ensure that adequate room exists in the waste bottle and adequate volume is available in the supply bottle **before** running AutoClean! |

The **Ultrasonic Advantage™** feature is a built-in **ultrasonic cleaner** that provides enhanced periodic maintenance capabilities by using ultrasonic pulses in a water bath to clean residuals from the manifold tubes. Ultrasonic energy causes cavitation forces within the water bath, which in turn cause tiny vapor bubbles to be created. The formation and subsequent collapse of these bubbles is the mechanism that cleans manifold tubes submerged in the bath.

The cleaner consists of a stainless steel reservoir with an ultrasonic transducer bonded to the bottom of the reservoir. The reservoir is mounted on the washer and also functions as the priming trough.

- **Do not** remove the ultrasonic cleaner! Only BioTek authorized service personnel should remove the ultrasonic cleaner for maintenance or repair.



or **WASHR>ACLEAN**

Running the **AutoClean** routine requires defining a protocol that includes a prime step and specifies the duration. Approximately **93 mL** of fluid fills the reservoir during each run.

While the program is running, the ultrasonic cleaner will pulse on and off approximately every ten seconds, and you will hear a periodic "hissing" sound that indicates the ultrasonic energy is present.

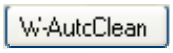
**Tip**: Detergent such as Terg-A-Zyme® added to deionized water in the supply bottle helps to break down the water's surface tension and enhances the cleaning process. Terg-A-Zyme also contains protease enzyme to digest protinaceous residue such as bovine serum albumin (BSA).

- **General Recommendation**: Run AutoClean for 1 hour. Follow with a full prime protocol using deionized water to remove the detergent from the system, or with a wash buffer to leave the instrument primed and ready for use. See the recommended prime volumes ***Chapter 3, Operation***.

❖ **1536-well hardware:** If you intend to AutoClean the 32-tube Syringe dispenser manifolds, we recommend cleaning them first, and then cleaning the 128-tube aspirate manifold. See below.

❖ **With the BioStack configured for keypad control**: Before running an AutoClean protocol when you have the BioStack connected to and controlled by the EL406, disable the BioStack until the process is complete. Review the details of this maintenance issue on page 107.

### To run AutoClean:

1. Empty the waste bottle.
2. Make sure supply bottles have sufficient volume of detergent and deionized water.
3. Create an AutoClean protocol, with these parameters:
   - First a **Prime** step to flush the lines with the cleaning agent.
   - 

**Add** an AutoClean step. **WASHR>ACLEAN**
   - Set the duration to 1 hour.
   -  **Save** the protocol and assign a unique name, e.g., AutoClean.
4. Click **Run**.

### Special Procedures for 1536-well Hardware:

EL406 models for processing 1536-well plates require your intervention to run **AutoClean**:

- Connect a bottle of cleanser or water to the **Dispense Fluid In** port on the rear of the instrument to use the single priming tube in the 128-tube aspirate manifold to fill the priming trough (ultrasonic bath).
- The 128-tube aspirate manifold cannot completely evacuate the priming trough. When AutoClean is finished, you must manually remove the residual liquid using a pipette, paper towel or other method. Be sure to follow the manufacturer's handling instructions for the cleaning fluid you are using.

### AutoClean 32-Tube Dispense Manifolds:

With a little manual intervention, you can use the ultrasonic cleaner on the hard-to-clean dispense tubes, especially the SB – small bore models.

**Before** running AutoClean for the aspirate manifold, run it for the 32-tube Syringe dispenser manifolds:

1. First, remove the Peri-pump cassette and put it aside.
2. Grasp both Syringe dispenser manifolds, and keeping them together, slide them off the dispense arm. The magnets help keep them together.
3. Gently release their tubing from the black bracket above the wash manifold and position them on the left side of the wash manifold, ready to put into the ultrasonic bath.
4. Run the AutoClean protocol described in the previous procedure.
5. When the AutoClean step begins, lift the wash manifold with your right hand and place the dispense manifolds into the bath with your left hand.
6. Make sure the tubes are in the fluid and the edges of the manifolds are resting on the edges of the priming trough. Then, gently lower the wash manifold down on top of the dispense manifolds. The wash manifold will hold them in place.

**Important**: the aspirate manifold will be unable to evacuate the fluid from the priming trough when the run ends because the dispense manifolds will be in the way. To complete the process:

7 Reinstall the dispense manifolds on the dispense arm.

8 Run the AutoClean protocol for the aspirate manifold.

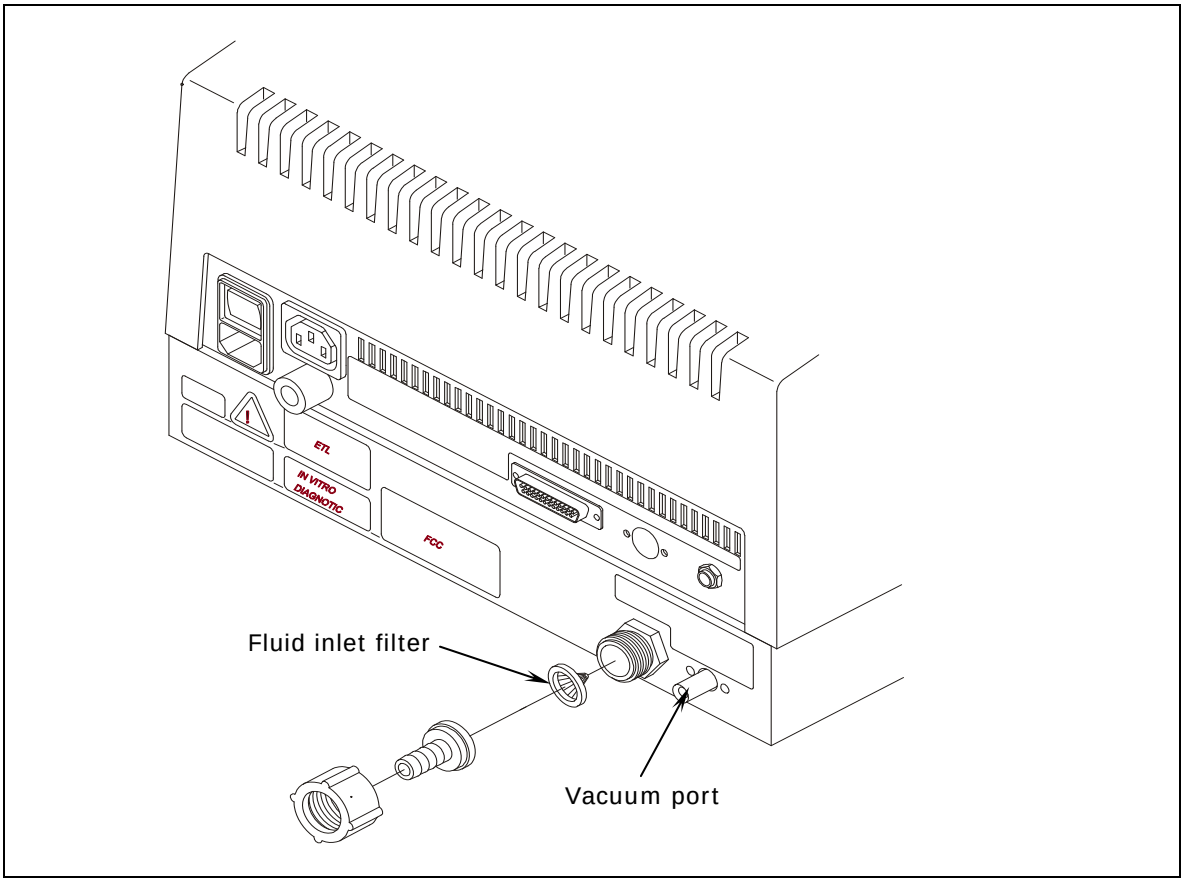
9 When AutoClean is finished, remove the residual liquid in the priming trough using a paper towel or other method. Be sure to follow the manufacturer's handling instructions for the cleaning fluid you are using.

## *Cleaning the Fluid Inlet Filter*

**Warning! Internal Voltage.** Turn off and unplug the instrument for all cleaning operations.

Periodically clean the fluid inlet filter (PN 49943):

1. Unscrew the **Dispense Fluid In** fitting from the back of the instrument.
2. Note the orientation of the filter in the fitting (the cone-shaped end of the filter points "in" toward the instrument) and then remove the filter from the fitting.
3. Wash the filter with hot water and a soft-bristled brush.
4. Rinse the filter, then replace it in the fitting and reinstall.



# Peri-pump Dispenser Maintenance

The level of the maintenance required to keep the Peri-pump performing as expected is dependent on several factors, including the type of fluid dispensed, the frequency of dispensing, and the work habits employed. For example, when dispensing fluids that can crystallize or harden after use, maintenance activities are required more frequently. Similarly, when using the 1 µL cassette, fluids should be filtered to 50 microns to reduce the chance of tips clogging.

Daily maintenance includes purging the fluid at the end of a dispense run and flushing the tubing with water (or buffered saline and then water). This is a good practice whenever the dispenser will be idle for more than an hour, as well as at the end of the day.

Another important daily requirement is keeping track of the number of plates processed with a cassette. This is necessary to determine when the cassette has reached its expected lifetime and is due for replacement or recalibration. Replacement Tubing Kits, as well as new cassettes are available from BioTek Instruments.

Monthly maintenance requires overall cleaning of the dispenser and its accessories, and verifying performance to determine if the cassette needs recalibration. Autoclaving or decontaminating the cassette is also recommended.

## Flush the Tubing Cassette

Daily Maintenance involves priming the tubing with an appropriate reagent at the beginning of the day, and, flushing the tubing to effectively remove all contaminants at the end of the day.

The type of rinse fluid to use is determined by the type of fluid you are dispensing. Some dispense fluids require the use of enzyme-active detergent, buffered saline, ethanol or isopropyl alcohol, rather than deionized water alone.

### Tools and Supplies

- Deionized or distilled water
- Buffered saline solution or enzyme-active detergent for protein or cell-based assays

### At the start of the day:

Prime the tubing to prepare for a dispense run.

1. Reload the cassette and fill the supply vessel:

- When dispensing solutions not effected by water, simply prime with the dispense fluid.
- When dispensing protein solutions, first prime the tubing with a buffered saline solution to remove any traces of water in the tubing, then, prime with the dispense fluid.

2. Hold the **Prime** button on the keypad until fluid flows into the priming trough and all visible air bubbles have been removed.

### At the end of the day:

Purge the tubing to reclaim the dispense fluid, then prime the tubing to flush it clean.

1. Hold the **Purge** button on the keypad until the tubing appears empty.
2. Replace the supply vessel with the appropriate rinse fluid:
   - When dispensing water soluble solutions, use DI water.
   - When dispensing protein solutions, first prime the tubing with a buffered saline solution to remove protein particles, then, prime with DI water.
3. Hold the **Prime** button on the keypad:
   - 1 µL cassette = 5 seconds
   - 5 µL cassette = 7 seconds
   - 10 µL cassette = 10 seconds.

## Unclog the Dispense Tips

The small diameter of the dispense tips makes them susceptible to clogging. You may be able to visually identify a clogged tip, or an inaccurate dispense performance may signal a problem. Good work habits can prevent clogging or reduce its occurrence:

- When using 1 µL cassettes, filter fluids to 50 microns before dispensing.
- Thoroughly flush the tubing after/in-between usage, especially when using liquids that crystallize or harden.

In case the need arises, BioTek ships a 10 cc plastic syringe with special tubing and fitting for use in unclogging tips. Installation instructions recommend storing it in the pouch on the back of the instrument. The remedy involves removing the dispense tip and flushing it with water. Depending on the type of clog, soaking the tip holder in hot water with mild detergent is recommended.

 This task may be easier if you use the cassette's shipping container to hold the unaffected cassette parts, keeping them out of your way.

### Required Materials

- 10 cc syringe with tubing and fitting attachment shipped with dispenser
- Screwdriver shipped with dispenser
- A sufficient quantity of deionized (DI) water in a beaker

### Procedure

1. Fill the 10 cc syringe with water and set aside.
2. Remove the cassette from the dispenser.
3. Use the screwdriver to open the **Tip Holder**. Put the top of the holder aside.
4. Lift the affected dispense tube from the holder and pull its tip off the tube.
5. Slide the tip, tapered end first, into the tubing on the end of the syringe.
6. With the tip poised to expel the clog and the water into the beaker or a sink, discharge the syringe.
7. Fill and discharge the syringe as many times as needed to flush the tip.
8. Reassemble the cassette:
    - Put the straight end of the tip into the bottom of the tube (the tapered end of the dispense tip is exposed).
    - Reinsert the tube into the Tip Holder. Seat the flared edges of the tip into the molded slots.

- Replace the Tip Holder cover with its two screws. The etched BioTek label identifies the top of the cover (except for 1536 cassettes' steel cover plate).

## Record the number of plates processed

To determine when a tubing cassette has reached the end of its expected lifetime, make a habit of counting and recording the approximate number of plates and volume dispensed per cassette.

Create a form similar to the example table below or estimate your usage of the cassette and project a date for replacement or recalibration.

| Cassette Types | Cassette Life | Total Volume |
|---|---|---|
| 1 µL | 1000 384-well plates @ 5 µL/well | 2,000 mL |
| 5 µL | 1000 96-well plates @ 50 µL/well | 5,000 mL |
| 10 µL | 1000 96-well plates @ 100 µL/well | 10,000 mL |

### Example table for recording cassette usage:

You may want to create a form similar to this table to keep track of the volume dispensed with each cassette:

| Cassette serial #: **2178** | | | | | |
|---|---|---|---|---|---|
| Date | # Plates | Plate Type | Volume/Well | Total Daily Vol. | Total Cassette Vol |
| 10/10/08 | 26 | 384 | 5 µL | 49920 µL | 50 mL |
| 10/11/08 | 33 | 96 | 10 µL | 31680 µL | 82 mL |
| | | | | | |

# Syringe Dispenser Maintenance

**32-tube small bore (SB) dispense manifold**: If you observe the fluid streaming from a dispense tube to be awry or skewed, it is most likely caused by minute particles of debris on the end of the tube. Brush away any particles from the end of the tube using a piece of silicon tubing. Silicon will not flake off and leave particles behind like other materials.

Also see **AutoClean the Dispense Manifolds** on page 120.

### Clean the Bottles and Tubes

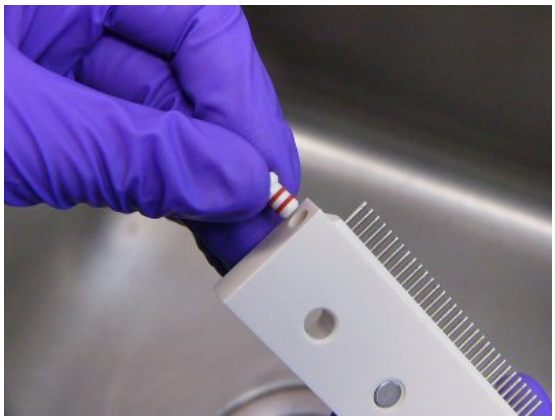
- Clean the bottles and tubing with deionized water before the first use, before each refill, and if they have been idle for any length of time.
- Accumulated algae, fungi, or mold may require decontamination; see page 131.

## Clean the Syringe Dispenser Manifolds

Regular rinsing helps to keep the manifold clean and the dispense tubing clear, and will increase the life of the tubing. Follow the Decontamination procedure starting on page 134 to disinfect the manifold and tubing.

If you suspect a particular problem is related to the manifold (for example, clogged tubes can result in uneven dispensing), you should perform a thorough cleaning of the manifold. To clean the manifold:

1. Turn off and unplug the instrument.
2. Pull each manifold off the dispense arm and disconnect the tubing from the syringe pump.
3. Using a lint-free disposable towel, thoroughly clean the outside of the dispense tubes.
4. Remove the plugs from the ends of the manifold.



5. Run hot water through the inlet fitting. Check to see if water comes out of all of the dispense tubes (except when working with the 32-tube SB manifold: there will be insufficient pressure to push water through the tubes). If not, soak the manifold in hot soapy water and repeat.

If additional cleaning is necessary, follow the procedure to clean the dispense tubes.

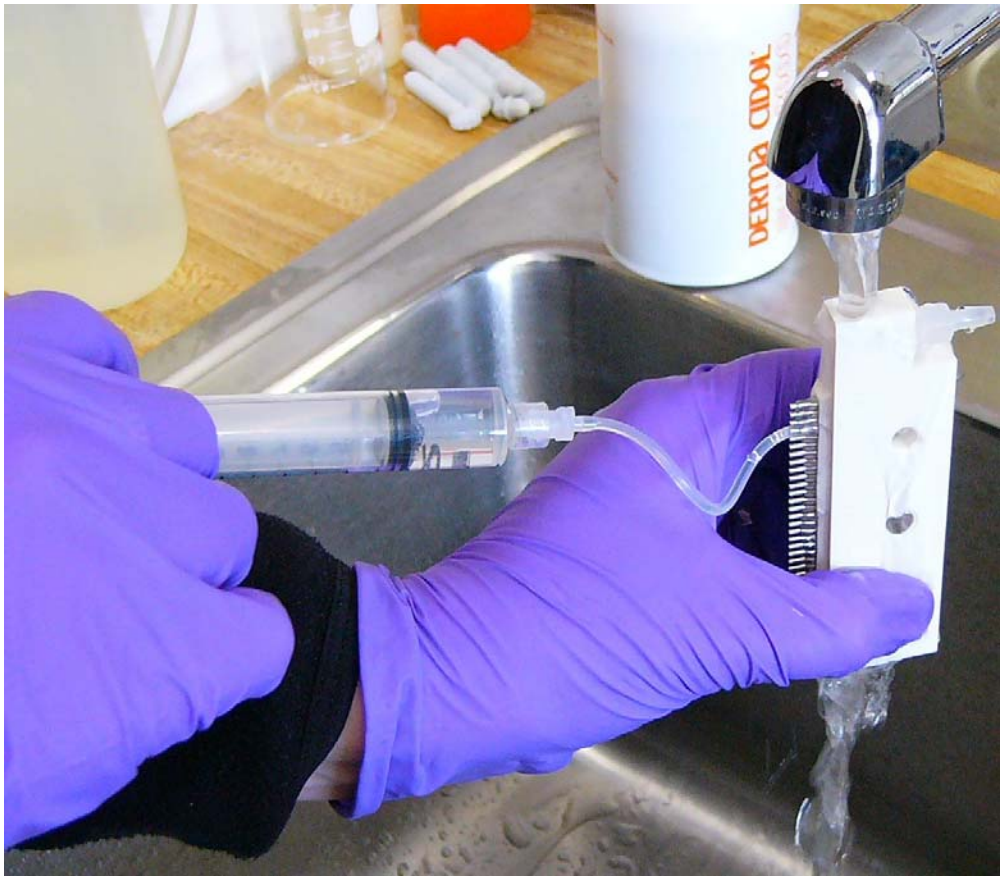
## Clean the Dispense Tubes

Unless there is a problem, the manifold dispense tubes do not need special cleaning. Periodic rinsing is usually sufficient to keep the tubes clean. However, if the previous steps are not completely successful, try the following:

- 16-tube manifolds: clean the tubes with the stylus;
- 32-tube manifolds: flush the tubing with the 10 cc syringe or clean the tubes with the stylus.

❖ **Note:** The 16-tube **autoclavable** manifold has removable dispense tubes. We do not recommend routinely removing these tubes. In the case of a particularly difficult problem with any one channel, however, a tube may be removed and cleaned individually, or replaced.

❖ **Do not autoclave the 32-tube dispense manifolds**!




1. Remove the two plugs from the ends of the manifold.
2. Tip the manifold on end and flush hot water through this open channel.
3. Using the supplied tool, clean the insides of the dispense tubes:
   - 16-tube manifold: use the stylus, PN 2872304
   - 32-tube manifold: use the 10 cc syringe and tubing or the appropriate stylus.

   Let water flow through the open channel while you probe or flush each tube, forcing any particles to be washed away.
4. Rinse the manifold with deionized or distilled water. Check to see if water comes out of all of the dispense tubes (except when working with the 32-tube SB manifold). Reinsert the plugs into the ends of the manifold.
5. Replace the tubing and remount the manifold.
6. Run a prime protocol, e.g. S_DAY_RINSE, using 40 mL of deionized water.
7. Verify dispense performance visually, or by performing the appropriate Dispense Precision and Accuracy Tests in ***Chapter 5, Qualification***.

## Clean/Replace the Check Valves

❖ **Note:** The check valves do not twist open.

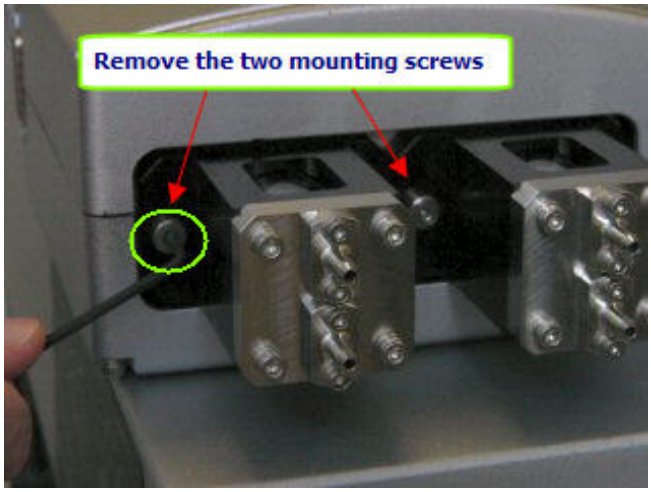
If the check valves leak or become clogged, you can either clean or replace them. Contact BioTek Customer Service to order replacement check valves.

To clean a check valve:

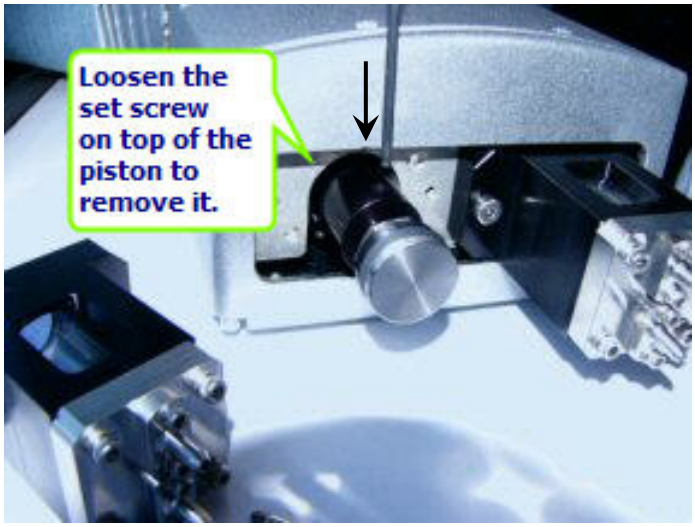
1. Pull the tubing off the check valve.
2. Insert the stylus into the feed end of the valve to hold it open (observe arrow on valve indicating flow direction).
3. Flush with hot water.
4. Replace the valve and the tubing.

After replacing a check valve, if you observe a decline in accuracy, **recalibrate the backlash** (see page 142) to ensure optimum performance.

## Autoclave the Syringe Head




1. Use the supplied 3/32″ (2.39 mm) hex wrench to remove the two mounting screws that hold the syringe head in unit.
2. Pull the syringe head straight back and off of the piston.




3. Use the hex wrench to loosen the setscrew on top of the sleeve that holds the piston and then remove the piston.

**Important!** Autoclave the piston and syringe head separated from one another. Keep the piston and syringe head unattached to each other when autoclaving.

4. Autoclave at 134°C and 216 kPa for 3 minutes, or 121°C and 115 kPa for 30 minutes. The manifold, tubing, autoclavable check valves, and supply bottles may also be sterilized in the autoclave.

❖ Check valves (PN 68073) recommended for use with organic substances cannot be autoclaved.

5. Replace the components by reversing the steps:
    1. With the flat side of the shaft facing up, slide the **syringe piston shaft** into the **piston holder** until it stops.
    2. Use the 3/32″ (2.39 mm) hex wrench to tighten the **setscrew**.
    3. Push the **syringe head** over the piston until it is flush with the unit and use the hex wrench to attach the two **mounting screws**.

# Decontamination

Any laboratory instrument that has been used for research or clinical analysis is considered a biohazard and requires decontamination prior to handling.

Decontamination minimizes the risk to all who come into contact with the instrument during shipping, handling, and servicing. Decontamination is required by the U.S. Department of Transportation regulations. Persons performing the decontamination process must be familiar with the basic setup and operation of the instrument.

The recommended **frequency for decontamination** is at least monthly, and before shipment of the instrument to BioTek for calibration or repair.

| | |
|---|---|
|  | **Important!** BioTek Instruments, Inc. recommends the use of the following decontamination solutions and methods based on our knowledge of the instrument and recommendations of the Centers for Disease Control and Prevention (CDC). Neither BioTek nor the CDC assumes any liability for the adequacy of these solutions and methods. Each laboratory must ensure that decontamination procedures are adequate for the Biohazard(s) they handle. |
|  | **Warning! Internal Voltage.** Turn off and unplug the instrument for all decontamination operations. |
|  | **Do not** immerse the instrument, spray it with liquid, or use a “wet” cloth. Do not allow the cleaning solution to run into the interior of the instrument. If this happens, contact the BioTek Service Department. **Do not soak the keypad.** |
|  | Wear prophylactic gloves when handling contaminated instruments. Gloved hands should be considered contaminated at all times; keep gloved hands away from eyes, mouth, nose, and ears. Eating and drinking while decontaminating instruments is not advised. |
|  | Mucous membranes are considered prime entry routes for infectious agents. Wear eye protection and a surgical mask when there is a possibility of aerosol contamination. Intact skin is generally considered an effective barrier against infectious organisms; however, small abrasions and cuts may not always be visible. Wear protective gloves when performing the decontamination procedure. |

## Tools and Supplies

- 0.5% sodium hypochlorite (NaClO, or bleach)
- 70% isopropyl alcohol (as a bleach alternative)
- Deionized or distilled water
- Priming plate
- Safety glasses
- Surgical mask
- Protective gloves
- Lab coat
- Biohazard trash bags
- Clean cotton cloths

## Autoclavable Components

Autoclaving is an efficient method of sterilizing instrument components. For qualified items, it is a good alternative to some of the following decontamination procedures.

| **Do autoclave:** | **Do NOT autoclave:** |
|---|---|
| Peri-pump cassettes | Washer manifolds |
| 16-Tube Syringe dispenser manifolds | 32-Tube Syringe dispenser manifolds |
| Syringe pump head | Plate carrier |
| All Syringe module tubing and bottles | |
| Priming trough inserts | |

## Decon Procedure for External Surfaces of the Instrument

| | |
|---|---|
|  | The bleach solution is caustic; wear gloves and eye protection when handling this solution. |
|  | **Caution!** Be sure to check the percent NaClO of the bleach you are using; this information is printed on the side of the bottle. Commercial bleach is typically 10% NaClO; if this is the case, prepare a 1:20 dilution. Household bleach is typically 5% NaClO; if this is the case, prepare a 1:10 dilution. |

1. Turn the instrument off and disconnect the power cord. Empty the waste bottle.
2. Unload the Peri-pump cassette and the prime trough inserts, and remove the Syringe dispenser manifold and tubing, if applicable.
3. Autoclave the cassette and Syringe dispenser components, including the priming trough inserts.

❖ **Do not autoclave the 32-tube dispense manifolds.** Review the list of non-autoclavable components above.

4 Prepare an aqueous solution of 0.5% sodium hypochlorite (NaClO, or bleach). As an alternative, 70% isopropyl alcohol (or 70% ethanol) may be used if the effects of bleach are a concern.

❖ **Isopropyl alcohol** is not recommended for removing **proteins** (such as bovine serum albumin).

5 Moisten a cloth with the bleach solution or alcohol. **Do not soak the cloth**.

- Wipe the keypad (do not soak). Wipe again with a clean cloth moistened with deionized or distilled water. Dry immediately with a clean, dry cloth.
- Remove the mist shield if it is attached. Wipe the inside and outside surfaces of the mist shield. Wipe the plate carrier, top surface of the instrument's base, supply bottles and tubing, and all exposed surfaces of the instrument.

6 Wait 20 minutes. Moisten a cloth with DI or distilled water.

- Wipe the inside and outside surfaces of the mist shield. Wipe the plate carrier, top surface of the instrument's base, supply bottles, tubing, bottle covers and all exposed surfaces of the instrument that have been cleaned with the bleach solution or alcohol.

7 Use a clean, dry cloth to dry all wet surfaces.

8 Reassemble the instrument as necessary.

9 Discard the used gloves and cloths using a Biohazard trash bag and an approved Biohazard container.

## Decon Procedure for Tubing and Manifold

Two pre-defined protocols are provided. These protocols flush and soak the supply tubing and manifolds with disinfectant, then flush the system with rinse fluid.

When using the LHC to control the instrument and with the Buffer Switching module installed, the washer protocols can be modified to run unattended. Otherwise, when using the LHC without the Buffer Switching module or when controlling the instrument from the keypad, manual intervention is required.

| Using the | Description |
|---|---|
| **Keypad:**<br>Protocols are loaded onboard at the factory. | • W-DECON (07) – for the washer<br>• S-DECON (08) – for the Syringe dispenser<br>1. At the main menu, press **RUN**.<br>2. Press **Options** to scroll to the desired protocol or use number keys to enter its number.<br>3. Press **ENTER** and follow the prompts. |
| **LHC**:<br>Protocols are installed on the host computer during installation. | • W-DECONTAMINATE – for the washer<br>• S-DECONTAMINATE – for the Syringe dispenser<br>1.  Select **Open** and locate the EL406 folder.<br>2. Open the EL406 folder to access the Maintenance folder.<br>3. Select the desired protocol. |

When storing or shipping the instrument, the pre-defined LONG_SHUTDOWN protocols prime and soak the instrument, and end by pushing air through the system. The parameters can be edited for optimum cleaning. For example, consider using ethanol instead of air to complete the decontamination process.

❖ Two supply bottles are required for this procedure: one for disinfectant, and one for rinse. Likewise for decontaminating the Syringe dispenser manifolds, the two supply bottles can be used for disinfectant and rinse.

1. Turn the instrument off and disconnect the power cord.
2. Empty the waste bottle.
3. Prepare an aqueous solution of 0.5% sodium hypochlorite (NaClO, or bleach).
4. Fill one supply bottle with at least 400 mL of bleach solution (disinfectant).
5. Fill another supply bottle with at least 800 mL of deionized water (rinse).

Preparing to run **W-DECON** or **W-DECONTAMINATE**:

| Using the | Description |
|---|---|
| **Keypad with Buffer Switching** | Connect the supply bottles this way:<br>• Buffer valve A: Disinfectant bottle<br>• Buffer valve B: Rinse solution bottle |
| **Keypad without Buffer Switching** | Connect the disinfectant bottle to the **Fluid In** port. When the protocol is finished, connect the rinse bottle to the inlet port, and press REPEAT to rerun the protocol. |
| **LHC with Buffer Switching** | Connect the supply bottles this way:<br>• Buffer valve A: Disinfectant bottle<br>• Buffer valve B: Rinse solution bottle<br>For unattended operation, change the Delay steps, make the delay a fixed time rather than indefinite. |
| **LHC without Buffer Switching** | Connect the disinfectant bottle to the **Fluid In** port.<br>At the prompt, when the protocol is delayed, connect the rinse bottle to the inlet port, and press **Continue** to complete the protocol. |

Preparing to run **S-DECON** or **S-DECONTAMINATE**:

| Using the | Description |
|---|---|
| **Keypad** | Connect the disinfectant bottle to the **Syringe A** port. When the protocol is finished, connect the rinse bottle to the port, and press REPEAT to rerun the protocol.<br>Make a copy of the protocol and modify the copy for **Syringe B**, e.g. SB-DECON, and repeat the above procedure. |
| **LHC without Buffer Switching** | Connect the disinfectant bottle to the **Syringe A** port. At the prompt, when the protocol is delayed, connect the rinse bottle to the inlet port, and press **Continue** to complete the protocol.<br>Copy the protocol, select **File>Save As**, assign it a unique name, e.g. SB-DECONTAMINATE, and modify it for **Syringe B**. Then, replicate the procedure. |

6. Reconnect the power cord and turn on the instrument.
7. Run the decontamination protocols.

❖ While the protocols are running, you will need to periodically check for prompts on-screen and follow the instructions.

## Alternate Decontamination Procedure for Tubing and Manifold

If you are unable to run the decontamination protocols due to a system failure, perform the following alternate decontamination procedure to disinfect the internal tubing and manifolds.

**Caution!** Be sure to check the percent NaClO of the bleach you are using; this information is printed on the side of the bottle. Commercial bleach is typically 10% NaClO; if this is the case, prepare a 1:20 dilution. Household bleach is typically 5% NaClO; if this is the case, prepare a 1:10 dilution.

1. Turn off the instrument and disconnect the power cord.
2. Remove the mist shield, if it is attached.
3. Using the 9/64" (3.57 mm) hex wrench, remove the screws, washers, and springs that hold the washer manifolds in place. Remove the manifolds.

❖ Hold the two manifolds (and end plates) together as a single unit when removing and replacing them (except for the 128-tube model).

4. Prepare an aqueous solution of 0.5% sodium hypochlorite (bleach). As an alternative, 70% isopropyl alcohol (or ethanol) may be used if the effects of bleach are a concern.

❖ **Isopropyl alcohol** is not recommended for removing **proteins** (such as bovine serum albumin).

5. Soak the tubing and manifold in the bleach or alcohol solution.
6. Wait 20 minutes. Rinse the tubing and manifold with DI or distilled water.
7. Use a clean, dry cloth to dry all wet surfaces of the tubes and manifold.
8. Reassemble the washer manifold, making sure that the two o-rings are in place prior to reassembly. **Do not over-tighten the manifold screws.**
9. Re-attach the mist shield.
10. Prime the system by running **W-DAY_RINSE** or a similar Maintenance protocol. Watch for leaks. If fluid leaks out of the back of the instrument, firmly seat the tubing. If fluid leaks from the manifold, try disassembling and carefully reassembling.
11. Discard the used gloves and cloths using a Biohazard trash bag and an approved Biohazard container.

**Caution:** When reinstalling the manifold, only tighten the screw-washer-spring assembly that holds it in place until you feel the mechanical stop. Tightening past this point will damage the instrument and **void your warranty.**

# Prepare for Storage or Shipment

Before the EL406 is shipped or stored, the entire system should be rinsed and soaked with disinfectant and then purged of all fluid. Perform these steps when leaving the instrument unused for a long period of time.

## Long_Shutdown

Two pre-defined protocols are provided:

- W-LONG_SHUTDOWN (09)– for the washer
- S-LONG_SHUTDOWN (10) – for the Syringe dispenser

These LONG_SHUTDOWN protocols flush and soak the supply tubing and manifolds with disinfectant, then flush with rinse, and finally purge the system of fluid.

❖ Three supply bottles are required for this procedure: one for disinfectant, one for rinse, and one for air.

**Caution!** Be sure to check the percent NaClO of the bleach you are using; this information is printed on the side of the bottle. Commercial bleach is typically 10% NaClO; if this is the case, prepare a 1:20 dilution. Household bleach is typically 5% NaClO; if this is the case, prepare a 1:10 dilution.

1. Turn the instrument off and disconnect the power cord.
2. Unload the Peri-pump cassette and the prime trough inserts. Clean and store them separately.
3. Empty the waste bottle.
4. Prepare an aqueous solution of 0.5% sodium hypochlorite (NaClO, or bleach).
5. Fill one supply bottle with at least 400 mL of bleach solution (disinfectant).
6. Fill another supply bottle with at least 800 mL of deionized water (rinse).
7. Keep the third supply bottle empty (air).

   When using the **external Buffer Switching module**, connect the supply bottles this way:

   - **Valve A:** Disinfectant bottle
   - **Valve B:** Rinse solution bottle
   - **Valve C:** Empty bottle

❖ When not using the Buffer Switching module, modify the protocol and connect the bottle containing disinfectant to the **Fluid In** port.

8 Turn on the washer and run W-LONG_SHUTDOWN (09).

9 When the washer has been cleaned and purged, repeat the process to clean and purge the Syringe dispensers, if applicable:

- Prepare the supply bottles with disinfectant, rinse and air;
- Run S-LONG_SHUTDOWN (10) three times for each manifold, one for each supply bottle.

## Storing the Washer Dispenser

After performing the **Long_Shutdown** protocols:

- Turn off the instrument and disconnect the power cord.
- Store it on a flat surface that is relatively free of vibration, in a dust-free and particle-free environment.
- Protect the instrument from temperature extremes that can cause condensation within the unit and from corrosive fumes and vapors.
- Store the instrument under the following environmental conditions:

  **Temperature:** –20° to 50°C (-4° to 122°F)

  **Relative humidity:** 10% to 85% (non-condensing)

❖ **Important:** Allow the instrument to reach room temperature before use after storage.

# Replace Components

Some components of the EL406 must be replaced periodically to maintain specified performance levels.

## Washer Components

Find instructions for changing the manifold in ***Appendix B***.

### *Replace Vacuum Pump Fuse*


Spare fuses (PN 46055) are shipped with the instrument in case the pump blows a fuse. To change the fuse:

1. Locate the **Accessory Fuse** port on the rear panel below the **Accessory Outlet** for the vacuum pump.
2. Use a screwdriver to open the port and release the fuse. It has a spring action.
3. Replace the fuse and reinstall.

## Washer O-Rings and Channel-End Seals

❖ Important: This is **not** applicable to the 128-tube aspirate manifold. The plugs in its aspirate manifold are the same as those used in the Syringe dispenser manifolds: PN 45090.

For optimal performance and to extend the life of the washer, replace the manifold o-rings once a year and replace the 12 (or 24) rubber plugs (seals) on the channel ends if they show signs of cracking or drying out. Order the replacement o-rings and seals from BioTek:

- O-rings: PN 49941
- Channel-end seals: PN 49486

You must remove the washer manifold to change the o-rings and seals, so these tasks work best in conjunction with the ***Washer Maintenance*** procedure on page 116.

### *Replacing the O-Rings*

After cleaning and rinsing the manifold:

1. Using your fingers or an appropriate tool, such as a very small, flat screwdriver, remove the two o-rings that are exposed when the manifold is removed.
2. Replace the used o-rings with new ones by fitting them into the grooved slots.

3. If you are NOT replacing the channel end-seals at this time, reinstall the manifold. **Do not over-tighten the manifold screws.**

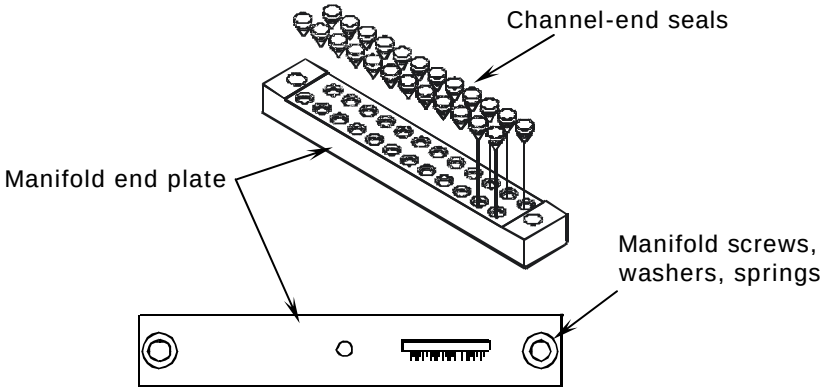
> **Caution:** When reinstalling the manifold, only tighten the screw-washer-spring assembly that holds it in place until you feel the mechanical stop. Tightening past this point will damage the instrument and **void your warranty.**

## *Replacing/Cleaning the Channel-End Seals*

❖ Important: This is **not** applicable to the 128-tube aspirate manifold.

If the channel-end seals do not need to be replaced, they should be washed with mild detergent or alcohol.

1. Remove the manifold end plates to access the channel-end seals.




Replacing the Channel-End Seals

The manifold end plate sits in front of the manifold and holds the screws, washers, and springs that hold the manifold in place. The channel-end seals sit in bored holes in the backside of the plate, facing the manifold's channels. The manifold end plate has markings to indicate its position relative to the manifold, e.g., Top or Bottom.

2. Using an appropriate tool, such as a very small, flat screwdriver or a paper clip, remove the seals from the manifold end plate.

> **Caution:** Do not grease any parts of this mechanism. Lubricate the seals and the bored holes with alcohol to assist with reinsertion.

3. Clean the seals if you are not replacing them with new ones.

4. Lubricate both the seals and the bored holes with 70% isopropyl alcohol to facilitate insertion of the seals. Make sure the seals sit firmly in the bored holes in the manifold end plate.

❖ The 9/64″ (3.57 mm) hex wrench shipped with the instrument is useful for reinserting the seals into the bored holes.

5. When all of the seals are in place, reinstall the manifold end plates and the manifold. **Do not over-tighten the manifold screws**.

**Caution:** When reinstalling the manifold, only tighten the screw-washer-spring assembly that holds it in place until you feel the mechanical stop. Tightening past this point will damage the instrument and **void your warranty.**

## Peri-pump Components

### *Replace Peri-pump Tubing*

BioTek provides replacement tubing kits as an alternative to buying a new cassette. Purchase the replacement tubing kits from BioTek and follow the instructions shipped with the kit or on the Operator's Manual CD in the PDF folder, titled: **7171017_(*current Rev*)_Replacing the tubing_8x14.PDF**. For the best experience with these instructions print them on legal size paper (8½″ x 14″).

### *Recalibrate the Peri-pump Dispense Cassette*

#### Calibration Kit

BioTek offers an accessory for recalibrating dispense cassettes. The Calibration Kit speeds up the recalibrating process and is useful for verifying performance, one of the recommended monthly maintenance routines.

Follow the instructions shipped with the kit or find them on the Operator's Manual CD in the PDF folder, titled: **7171009_(*current Rev*)_Calibration Kit Instructions.PDF**.

#### Gravimetric Method

The alternative and most precise method for calibrating a cassette is the gravimetric method. Find the instructions on the Operator's Manual CD in the PDF folder, titled: **7171024_(*current Rev*)_Calibrating Gravimetrically.PDF**

## Syringe Dispenser Components

### *Replace Syringe Dispenser Check Valve*

You can order replacement check valves from BioTek Customer Care if your check valves become clogged and cleaning them does not solve the problem:

- PN 68083 – Autoclavable valves for use with non-organic substances.
- PN 68073 – Check valves recommended for use with organic substances.

❖ If you observe a decline in performance after changing the check valves, recalibrate the backlash, as described below.

### *Recalibrate the Backlash*

❖ **Important Prerequisite**: The EL406 Interface Software (IS) is required to update the instrument's backlash setting. You must be controlling the EL406 with the LHC software or obtain a copy of the EL406 IS Utility from BioTek's customer resource center: https://customer.biotek.com.

❖ **1536-well models**: All Syringe dispensers are calibrated for use with their 16-tube dispense manifolds. When dispensing with the 32-tube dispensers, use this procedure to determine if a backlash adjustment improves dispense accuracy.

#### Equipment Required

- Precision balance with minimum capacity of 100 g and readability of 0.001 g resolution
- Supply bottle with deionized water
- 384-well plate for 16-tube manifolds or 1536-well plate for 32-tube manifolds (acceptable substitute: cover of 96-well microplate)

#### Setup

While calibrating and testing, try to maintain a steady liquid level in the supply bottle, keeping it half full. Start with more fluid to allow for priming. Connect the supply bottle to the Syringe under test. **Make sure the supply bottle is at the same level as the dispenser.**

#### Create a Dispense Protocol

Create a protocol that dispenses the correct volume for the manifold under test:

- **16 tube manifold: 20 µL** per well to a **384-well** plate at Flow Rate **2**.
- **32 tube manifold: 6 µL** per well to a **1536-well** plate at Flow Rate **2**.

### Prime and Dispense

1. Run a prime protocol, for example, **S-DAY_RINSE**, or **S-DAY_RINSE_A&B** when both Syringe pumps need calibration, to remove air bubbles from the tubing.
2. Place the plate on the balance, and tare the balance.
3. Place the plate onto the carrier and run the dispense protocol created above.
4. Upon completion, carefully remove and reweigh the plate to determine the **Actual Weight**.
5. The **Expected Weight** is:
    - 16-Tube = 7.680 grams
    - 32-Tube = 9.216 grams
6. Calculate the volume (weight) error and the backlash adjustment that needs to be made:

    **16-Tube: 7.680 – Actual Weight ÷ 24 ÷ .0033 = backlash setting**

    **32-Tube: 9.216 – Actual Weight ÷ 48 ÷ .0033 = backlash setting**

    - Subtract the Actual Weight from the Expected Weight and divide by the number of columns dispensed to.
    - Divide the result by 0.0033 and round to the nearest whole number.

      Example: 7.680 - 7.550 = 0.130, 0.130/24 = 0.00542, 0.00542/0.0033 = 1.64 rounded to 2. The backlash setting needs to be adjusted by 2.
7. Adjust the backlash as necessary: select **Tools> Instrument Utilities> Syringe Dispenser** and enter the number of steps in the corresponding **Backlash** fields in the Calibration Data group box.
8. Repeat steps 1 through 4 until the volume dispensed is within one backlash unit of being exact: ± 0.119 grams**.**

Chapter 5

# Qualification

This chapter describes how to verify the performance of the EL406 Washer Dispenser.

# Overview

Instrument verification for the EL406™ involves three activities: qualification of installation and setup, qualification of routine capability, and qualification of long-term stability. This chapter refers to these activities as Installation Qualification **(IQ)**, Operational Qualification **(OQ)**, and Performance Qualification **(PQ)**, respectively.

Verification testing includes:

- The **System Self Test** verifies system components, such as the vacuum, manifold, and carrier positioning. The **Checksum Test** verifies the basecode software against internal checksum values to ensure that no corruption has occurred.

### Washer

- **Evacuation Efficiency:** This test measures the residual volume per well after aspiration. The lower the residuals per well, the better the evacuation efficiency of the washer.
- **Dispense Precision:** This test measures the variability of volumes dispensed from tube to tube across the manifold.
- **Buffer Switching Valve Module:** This annual test measures the variability of volumes dispensed from tube to tube across the manifold when using the buffer switching module.

### Peri-pump and Syringe Dispensers

- **Dispense precision** is a measure of the variability of volumes dispensed from tube to tube across the manifold or tip holder. The optical density of the solution in a well is proportional to the total volume of the solution in the well. When the % Coefficient of Variation (%CV) is calculated, the result is a measure of the uniformity of the distribution of dispensed volumes across the manifold. It is the ratio, expressed in percent, of the standard deviation of the distribution of fluid volumes in the wells to the mean value of volume per well. The uniformity of distribution across the manifold improves as the %CV is lowered.
- **Dispense accuracy** is a measure of the average volume dispensed per well. It is independent of precision. The volume per well may vary greatly over a plate, yet the accuracy may be exact because it is an average of the volumes.

# Verification Schedule

The following schedule defines the factory-recommended intervals for verification tests for an instrument used two to five days a week. The schedule assumes that the EL406 is properly maintained as outlined in ***Chapter 4, Preventative Maintenance.***

❖ **Note:** An instrument qualification package (PN 7180527) is available for purchase. The package contains thorough procedures for performing Installation Qualification, Operational Qualification and Performance Qualification (IQ-OQ-PQ) and preventative maintenance (PM). Extensive Checklists and Logbooks are included for recording results. Contact your local dealer for more information.

### Recommended Instrument Verification Schedule

| Tests | IQ | OQ | | PQ |
|---|---|---|---|---|
| | Initially | Initially | Annually | Monthly |
| Unpacking, Installation, and Setup | ✓ | | | |
| Verify/Enable System Sensors | ✓ | | | ✓ |
| System Self Test and Checksum Test | ✓ | | ✓ | ✓ |
| Shake Test | | ✓ | ✓ | |
| Ultrasonic Cleaner Test | | ✓ | ✓ | |
| Washer Evacuation Efficiency Test | | ✓ | ✓ | ✓ |
| Washer Dispense Precision Test | | ✓ | ✓ | ✓ |
| Buffer Switching Dispense Test | | ✓ | ✓ | |
| Peri-pump Dispense Precision and Accuracy Test | | ✓ | ✓ | ✓ |
| Syringe Dispense Precision and Accuracy Test | | ✓ | ✓ | ✓ |
| Run Assay | | ✓ | ✓ | ✓ |

**Important!** The risk factors associated with your assays may require that the Operational and Performance Qualification procedures be performed more or less frequently than shown above.

# IQ/OQ/PQ

**Installation Qualification (IQ)** confirms that the EL406 and its components have been supplied as ordered and ensures that they are assembled and configured properly for your lab environment.

- The recommended IQ procedure consists of setting up the instrument as described in ***Chapter 2, Installation*** and then performing the System Self-Test and the Checksum test.
- The IQ procedure should be performed *initially* (before the washer is used for the first time).
- The successful completion of the IQ procedure verifies that the instrument is installed correctly. The Operational Qualification procedure (see below) should be performed immediately following the successful IQ.

**Operational Qualification (OQ)** confirms that the EL406™ operates according to specification initially and over time.

- The recommended OQ procedure consists of performing the System Self-Test and the Checksum, Evacuation Efficiency, and Dispense Precision and Accuracy tests.
- Your facility's operating policies may also require you to perform an actual assay prior to accepting the instrument for routine use. Do not use the data obtained from the first assay until you have confirmed that the assay kit criteria have been met.
- The OQ procedure should be performed *initially* (before first use) and then routinely; the recommended interval is *annually*. It should also be performed after any major repair or upgrade to the hardware or software.
- Although out-of-tolerance failures will be detected by the OQ tests, results should be compared with those from the monthly Performance Qualification tests and previous OQ tests to monitor for trends.
- The successful completion of the OQ procedure, in combination with results that are comparable to previous PQ and OQ tests, confirms that the instrument is performing consistently over time.

**Performance Qualification (PQ)** confirms that the EL406 consistently meets the requirements of the tests performed at your laboratory.

- The recommended PQ procedure consists of performing the System Self-Test and the Checksum, Evacuation Efficiency, and Dispense Precision tests.
- Your facility's operating policies may also require that you routinely perform an actual assay, to confirm that the instrument will consistently give adequate results for the assays to be run with it.
- These tests should be performed routinely; the recommended interval is *monthly*. This frequency may be adjusted depending on the trends observed over time.
- The successful completion of the PQ procedure confirms that the instrument is performing consistently under normal operating conditions.

# Important Recommendations for All Liquid Tests

### Test Solutions

- Using pure deionized water in place of the test solutions, e.g., **Solution #1**, is ***not*** recommended and will likely result in the failure to meet specifications.
- Prepare the solutions **the day before** you plan to run the tests. This will allow any foam caused by the agitation of solutions containing Tween® 20 to settle.
- BioTek determined the pass/fail specifications for the instrument tests using the recommended test solutions. You may use **your own buffer solution** instead, but if any tests fail using your own buffer, retry the tests using the recommended solutions.

### Plate Reading

- If you are using one of BioTek's keypad-based readers, such as the ELx800™ or ELx808™, ensure that the reader is **not** running in **Rapid mode**. To check the setting, select **UTIL → READ** and cycle through the options until READ IN RAPID MODE? appears. Set it to **NO**.
- The absorbance of blue dye solutions should be measured at 630/450 (or 405) nm. The BioTek blue dye solution part number is **7773001**.
- The absorbance of yellow dye solutions should be measured at 450 (or 405)/630 nm. The BioTek yellow dye solution part number is **7120782**.
- The final absorbance for all dye solution concentrations should be in a range between 0.700 and 1.300 OD.

### Recording Test Results

- Liquid Test Worksheet templates can be found at the end of each test's instructions for recording data reduction results. If your tests are failing, this information will be useful for BioTek TAC to help diagnose any problems.

# System and Checksum Tests

The information from the System and Checksum Tests is important if you need to contact BioTek.

The **System Self-Test** is performed automatically whenever the instrument is powered on. They can also be run manually.

## System Self-Test

The System Self-Test checks the vacuum (when run manually), manifold, and manifold-to-carrier positioning.

| Using the | Description |
|---|---|
| **Keypad** | 1. Press **Setup Menu**. (Setup Menu)<br>2. → Select the arrow.<br>3. Select **TESTS**.<br>4. Select **SLFCHK**. |
| **LHC** | 1. Select **Tools > Instrument Utilities**.<br>2. Under "General Settings," click **Perform Self-check.** |

**If the test passes**, a passing message is displayed.

**If the test fails**, an error message is displayed. Look up the error code in ***Appendix A, Error Codes***, to determine its cause. If the problem is something you can fix, turn off the EL406, fix the problem, and then turn instrument back on. If the cause is not something you can fix, contact BioTek's Technical Assistance Center.

## Checksum Test

The Checksum Test compares the on-board software with an internally recorded checksum value to ensure the program has not become corrupted.

**To run the Checksum Test:**

| Using the | Description |
|---|---|
| **Keypad** | 1. Press **Setup Menu**. (Setup Menu)<br>2. → Select the arrow (for more options) twice.<br>3. Select **TESTS**.<br>4. Select **CHKSUM**.<br>5. Reveal and record the values one at a time, **UI** and **MC**. |

| | |
|---|---|
| **LHC** | 1. Select **Tools > Instrument Utilities**.<br>2. Under "General Settings," find the **EL406 Basecode Software Information** group box and record the<br>• Software version and part number (e.g. PN 7180201),<br>• Data version,<br>• **UI** and **MC** Checksum values. |

# Shake Test

Perform this test to verify that the instrument's plate shaker is operating properly.

1. Create a simple protocol with a Shake step. Define a brief Shake Duration and choose any intensity.
2. Run the protocol. While it is running, visually verify that the plate shakes for the specified duration.
3. Verify that the program completes without error.

   If the plate shaker does not operate as expected, contact BioTek for assistance.

# Ultrasonic Cleaner Test

Perform this test to verify that the cleaner is operating properly.

1. Fill the supply bottle with one liter of deionized water.
2. Create a protocol with an **AutoClean** step. Set the **Duration** to **00:02** (2 minutes).
3. Run the protocol.
4. While the AutoClean program is running, the ultrasonic cleaner should pulse on and off *approximately* every ten seconds (a 50% duty cycle). While the program is running, listen for the periodic "hissing" sound that indicates the ultrasonic energy is present. Detecting the sound is all that is required to verify that the ultrasonic cleaner is operating properly. After two minutes, the washer will aspirate the fluid and the program will be complete.

❖ **Special Procedures for 1536-well Hardware**:
~ Connect a bottle of cleanser or water to the **Dispense Fluid In** port on the rear of the instrument to use the single priming tube in the 128-tube aspirate manifold to fill the priming trough (ultrasonic bath);
~ The 128-tube aspirate manifold cannot completely evacuate the priming trough. When AutoClean is finished, manually remove the residual liquid using a pipette, paper towel or other method.

If the ultrasonic cleaner does not operate as described above, contact BioTek for assistance.

# Washer Liquid Tests

## Evacuation Efficiency Test

The Evacuation Efficiency test measures the **residual volume** (mean residual weight) per well after aspiration is performed. The lower the residual per well, the better the evacuation efficiency of the washer. A known solution is dispensed into all wells of a previously weighed microplate. Aspiration is performed and the plate is reweighed. The total residual fluid is calculated based on the weight difference, and this value is divided by 96, 384 or 1536, as appropriate, to obtain the **mean residual weight**.

If further testing is necessary (to identify "problem" wells), the supplemental test is performed. A known dye concentration is dispensed to and evacuated from the wells, and the plate is weighed. Buffer is then pipetted to all wells to bring the volume of fluid to a more optically measureable volume. The optical density (OD) of each well is measured and the background is subtracted to account for scratches on the plate or particulates in a well. Each well's residual volume is calculated using its OD and a calibration factor derived from the mean residual weight and the mean OD of all wells on the plate. It is assumed that 1 mg = 1 µL of fluid for this calculation.

## Dispense Precision Test

The Dispense Precision Test measures the **variability of the volumes dispensed** from tube to tube across the manifold. In this test, a blue dye solution is dispensed into a microplate. The optical density of each well is measured at 630 nm and the background at 450 nm is subtracted to account for scratches on the plate or particulates in the well. The average error percentage is calculated and the amount dispensed to each well is calculated. Acceptance is based on the **%CV** (%Coefficient of Variation), or the ratio of the standard deviation of the distribution of fluid volumes in the wells to the mean value of volume per well. The lower the %CV, the better the uniformity across the manifold.

**Annual Buffer Switching Module Test:** The Dispense Precision test is conducted for the external buffer module to ensure that each valve (A, B, C, D) is calibrated to deliver the same volume of fluid.

## Manifold-Specific Tests

Perform the tests applicable to the installed **manifold type**. And, for washers equipped with the external **buffer switching module**, there is an annual test for each valve:

| Manifold Type | Liquid Tests to Perform | Page |
|---|---|---|
| **96-tube** | Evacuation Efficiency Test (96) | 157 |
| | Dispense Precision Test (96) | 157 |
| **192-tube** | Evacuation Efficiency Test (192) | 160 |
| | Dispense Precision Test (192) | 160 |
| **128-tube** | Evacuation Efficiency Test (1536) | 164 |
| | | |
| **Buffer Switching Module** | Annual Buffer Valve Test | 163 |

## Pre-defined Test Protocols

Several EL406 protocols are shipped onboard the instrument and with the **EL406 Interface Software (IS)**, which installs them on your computer during LHC installation. They include the protocols required to perform the Liquid Tests:

| Pre-defined Protocols | Keypad Number |
|---|---|
| **W-DAY_RINSE** | **01** |
| **W-96_EVAC_TEST** | **16** |
| **W-192_EVAC_TEST** | **18** |
| **W-1536_EVAC_TEST** | **19** |
| **W-96_DISP_TEST** | **15** |
| **W-192_DISP_TEST** | **17** |

- 1536-well washing using the 32- tube Syringe dispenser to dispense fluid. Perform the **Syringe dispenser tests** to verify dispense accuracy and precision. Then, use the same plate to test Evacuation Efficiency.

## Default Parameters for the Liquid Test Protocols

| Evacuation Efficiency Tests | | | |
|---|---|---|---|
| | **W-96_EVAC_TEST** | **W-192_EVAC_TEST** | **W-1536_EVAC_TEST** |
| Aspirate Height (Z) | 28 (3.556 mm) | 16 (2.03 mm) | 40 (5.08 mm) |
| X Position | -50 (-2.29 mm) | 00 (0 mm) | 00 (0 mm) |
| Y Position | 2 (0.15 mm) | 00 (0 mm) | 00 (0 mm) |
| Travel Rate | 2 (3.4 mm/sec) | 3 (4.0 mm/sec) | 3 (4.0 mm/sec) |
| Delay | 0 | 0 | 0 |
| Secondary Aspirate | No | No | No |

| Dispense Precision Tests | | | |
|---|---|---|---|
| | **W-96_DISP_TEST** | **W-192_DISP_TEST** | **S-1536_DIS_TEST** |
| Buffer Valve | A | A | See Syringe dispenser tests beginning on page 185. |
| Dispense Volume | 300 µL/well | 80 µL/well | |
| Flow Rate | 6 | 7 | |
| Dispense Height (Z) | 120 | 120 | |
| X Position | 00 | 00 | |
| Y Position | 00 | 00 | |
| Pre-dispense | No | No | |

## Required Materials

- One **new** microplate per test to be performed:

| Microplate Type | Liquid Tests |
|---|---|
| Flat-bottom 96-well plates, Corning® Costar #3590 or equivalent | Evacuation Efficiency Test (96) |
| | Dispense Precision Test (96) |
| | Annual Buffer Valve Test |
| | |
| Flat-bottom 384-well plates, Corning Costar or equivalent | Evacuation Efficiency Test (192) |
| | Dispense Precision Test (192) |
| | Annual Buffer Valve Test |
| | |
| Standard 1536-well plates, Nunc® #264710 or equivalent | Evacuation Efficiency Test (128) |

- Precision balance with minimum capacity of 100 g and readability of 0.001 g resolution
- Pipettes and graduated beakers
- Microplate absorbance reader capable of dual wavelength reading at 630/450 nm
- Liquid Test Worksheets at the end of this chapter for recording data and results
- Deionized water
- **Test solutions:**

| If this manifold is installed: | Prepare these solutions, using the instructions on the next page: | | | |
|---|---|---|---|---|
| | Sol. #1 | Sol. #2 | Sol. #3 | Sol. #4 |
| 96-Tube | 1 batch (1000 mL) | 1 batch (100 mL) | 1 batch (1200 mL) | n/a |
| 192-Tube | 1 batch (2000 mL) | 1 batch (100 mL) | n/a | 1 batch (1440 mL) |
| 128-Tube | 1 batch (100 mL) | | | |

❖ These volumes are sufficient for performing the Dispense Precision Test, the Evacuation Efficiency Test, and the additional Evacuation Diagnostic Procedure (if necessary). In most cases, enough fluid will be left over to re-run a test, if necessary.

- If you will be performing the **Annual Buffer Switching Test**, you will need several additional liters of DI water.

### *Test Solutions*

| **SOLUTION #1: Buffer Solution** | | |
|---|---|---|
| Pipette 1 mL Tween 20® into 1 liter (1000 mL) of deionized water and mix well. | **or** | Pipette 10 mL of BioTek Wetting Agent* into 1 liter of deionized water and mix well. |

** BioTek Solution #1 100X Concentrate Wetting Agent 125 mL (PN 7773002) contains 10% Tween 20 in deionized water and 0.01% Sodium Azide as a preservative.*

| **SOLUTION #2: Residual Test Solution** | | |
|---|---|---|
| Mix 100 mL of **Solution #1** with 0.050 grams of FD&C #1 blue dye. | **or** | Mix 90 mL of **Solution #1** with 10 mL of BioTek Blue Test Dye*. |

** BioTek Solution #2 10X Concentrate Blue Test Dye 125 mL (PN 7773001) contains 5 g per liter FD&C Blue #1, 0.1% Tween 20 in deionized water and 0.01% Sodium Azide as a preservative.*

| **SOLUTION #3: Dispense Precision Solution** for 96-Tube manifolds |
|---|
| Mix 1180 mL of deionized water with 20 mL of **Solution #2**. |

| **SOLUTION #4: Dispense Precision Solution** for 192-Tube manifolds |
|---|
| Mix 1420 mL of **Solution #1** with 20 mL of **Solution #2**. |

## Dispense Precision Test (96)

- This test is designed for **96-Tube** manifolds. See page 160 if you're looking for the Dispense Precision Test for the 192-Tube manifold.
- See page 155 for a list of required materials.
- When this test is complete, the dispensed plate can be used to perform the Evacuation Efficiency Test (see page 158).

1. Fill a supply bottle with 2 liters of DI water; Buffer Switching units use **Buffer valve A**. Run the **W-DAY_RINSE** protocol two or three times to prime the fluid lines and manifold.
2. Fill a supply bottle with 1200 mL of **Solution #3**; Buffer Switching units use **Buffer valve A**.
3. Run **W-DAY_RINSE** again to prime the washer with the solution.
4. Place a new 96-well microplate on the carrier and run the **W-96_DISP_TEST**. This protocol dispenses 300 µL of solution to each well of the plate. It does not evacuate the solution.
5. When the program is completed, carefully remove the plate.
6. Read the plate in an optical reader (blank on air), using the dual-wavelength method (630 nm - 450 nm), then print or export the results.
7. Using the *Dispense Precision Test Worksheet* on page 166, perform data reduction:
   a. Calculate the **Standard Deviation**.

      ***Tip:*** If you have a spreadsheet software program, enter/export all 96 values into a spreadsheet and apply your program's Standard Deviation function (e.g., Microsoft® Excel's STDEV).

   b. Calculate the sum of the OD values for all 96 wells, then divide by **96** to determine the **Mean OD** for the plate.

   c. Calculate the **%CV**: (Standard Deviation / Mean OD) * 100.

      The %CV should be **<= 3.0**.

      If the %CV is *greater than* 3.0, one or more dispense tubes may need to be cleaned. Run **AUTOCLEAN**, and/or remove the manifold and use the stylus to clean the dispense tube(s) giving lower-than-average absorbance readings. When finished, re-prime the washer and retry the test.
8. When finished, prime with deionized water to flush out the dye solution.

## Evacuation Efficiency Test (96)

- This test is designed for **96-Tube** manifolds. See page 160 if you're looking for the Evacuation Efficiency Test for the 192-Tube manifold.
- If you tared the balance at the start of the Dispense Precision Test (96), the plate from that test can be used here; **skip steps 1-3**.

1. Fill a supply bottle with 2 liters of DI water. Run the Maintenance program **W-DAY_RINSE** two or three times to prime the tubing and manifold.
2. Place a new 96-well microplate on the balance and zero the balance.
3. Pipette or dispense 300 µL of **Solution #1, 2, or 3** (your choice) into each well of the microplate.
4. Place the plate on the carrier and run the **W-96_EVAC_TEST** protocol. This protocol evacuates the wells, leaving a small amount of residual fluid.
5. When the program is completed, remove the plate and weigh it immediately because evaporation will affect the results. This is the **Total Residual Weight,** in grams.
6. Visually inspect the plate and note if any wells appear to have considerably more liquid than others.
7. Using the worksheet on page 167, perform initial data reduction:
   - Divide the Total Residual Weight by **96** to find the **Mean Residual Weight**.
   - The Mean Residual Weight should be **<= 0.002 g**.

If the Mean Residual Weight is *greater than* 0.002 g, or if one or more wells appear to have much more liquid than the others, the washer failed the test.

If the test fails once, **Troubleshoot as follows:**

- If the problem appears to be related to particular wells, clean those aspiration tubes: run **AutoClean**, and/or remove the manifold and thoroughly clean the tubes with the stylus (see ***Chapter 5, Maintenance*** for instructions.) When finished, retry the test.
- Failure of this test is commonly caused by improper aspiration tube positioning, usually because a microplate other than the recommended Corning Costar® 96 was used. If you prefer to use a different plate, modify the **Aspirate Positioning**, i.e., Z-axis to fix the height, or X- or Y-axes parameters in a copy of the **W-96_EVAC_TEST** protocol. After making needed changes, retry the test using a new microplate.

  If you *are* using the Corning Costar 96 microplate, run **AutoClean** and/or remove the manifold and clean the aspiration tubes with the stylus. When finished, retry the test using a new microplate.
- If the test fails a second time: perform the diagnostic procedure below.

## *Evacuation Diagnostic Procedure*

*Perform these steps if the Evacuation Efficiency Test fails twice. This test will confirm which aspirate tube(s) may be clogged, or if the plate's alignment or position is the problem.*

1. If you have not already done so, repeat steps 2 through 7 of the previous test, using **Solution #2** for the dispense fluid. Be sure to recalculate the **Mean Residual Weight**.
2. Pipette or dispense 300 µL of **Solution #1** into each well, on top of the residual solution.
3. Shake the plate to achieve uniform distribution of the remaining dye in each well.
4. Read the plate in an optical reader (blank on air), using the dual-wavelength method (630 nm - 450 nm), then print or export the results.
5. Using the worksheet on page 167, perform data reduction:
   - Calculate the sum of the OD values for all 96 wells, then divide by **96** to determine the **Mean OD** for the plate.
   - Divide the Mean OD by the **Mean Residual Weight** (from step 1), to find the **Residual Factor.**
   - For each well, divide its OD value by the Residual Factor to find its **Residual Weight.**

     Each well's Residual Weight should be **<= 0.002 g.**

   If one or more wells have a Residual Weight *greater than* 0.002 g, review the data to determine which well, or wells, is causing the problem.

   - ➢ If the problem appears to be related to particular wells, clean the associated aspiration tubes: run **AutoClean** and/or remove the manifold and thoroughly clean the tubes with the stylus. See ***Chapter 4, Maintenance*** for complete instructions. When finished, retry the test.
   - ➢ If the problem appears to be related to a particular region, edge, or corner of the plate, review the alignment and flatness of the plate on the carrier.

- **Please do not adjust** the carrier adjustment screws! Contact your BioTek dealer if you suspect an alignment problem.

   - ➢ For additional suggestions, see ***Chapter 6, Troubleshooting***.
   - ➢ If the test continues to fail, contact BioTek Instruments.

## Dispense Precision Test (192)

- This test is designed for the **192-Tube** manifold. See page 157 if you're looking for the Dispense Precision Test for 96-Tube manifolds.
- See page 155 for a list of required materials.
- When this test is complete, the dispensed plate can be used to perform the Evacuation Efficiency Test (see page 161).

1. Fill a supply bottle with 2 liters of DI water; Buffer Switching units use **Buffer valve A**. Run the **W-DAY_RINSE** protocol two or three times to prime the fluid lines and manifold.
2. Fill a supply bottle with 1440 mL of **Solution #4**; Buffer Switching units use **Buffer valve A**.
3. Run **W-DAY_RINSE** again to prime the washer with the solution.
4. Place a new 384-well microplate on the carrier and run the **W-192_DISP_TEST** protocol. This protocol dispenses 80 µL of solution to each well of the plate. It does not evacuate the solution.
5. When the program is finished, carefully remove the plate.
6. Read the plate in an optical reader (blank on air), using the dual-wavelength method (630 nm - 450 nm), then print or export the results.
7. Using the *Dispense Precision Test Worksheet* on page 169, perform data reduction:
   a. Calculate the **Standard Deviation**.

      ***Tip:*** If you have a spreadsheet software program, enter/export all 384 values into a spreadsheet and apply your program's Standard Deviation function (e.g., Microsoft Excel's STDEV).

   b. Calculate the sum of the OD values for all 384 wells, then divide by 384 to determine the **Mean OD** for the plate.

   c. Calculate the **%CV**: (Standard Deviation / Mean OD) * 100.

      The %CV should be **<= 4.0**.

      If the %CV is *greater than* 4.0, one or more dispense tubes may need to be cleaned. Run **AutoClean** and/or remove the manifold and use the stylus to clean the dispense tube(s) giving lower-than-average absorbance readings. When finished, re-prime and retry the test.
8. When finished, prime with deionized water to flush out the dye solution.

## Evacuation Efficiency Test (192)

- This test is designed for the **192-Tube** manifold. See page 157 if you're looking for the Evacuation Efficiency Test for 96-Tube manifolds.
- See page 155 for a list of required materials.
- If you tared the balance at the start of the Dispense Precision Test, the plate from that test can be used here; **skip steps 1-3**.

1. Fill a supply bottle with 2 liters of DI water. Run the **W-DAY_RINSE** protocol two or three times to prime the fluid lines and manifold.
2. Place a new 384-well microplate on the balance and zero the balance.
3. Pipette or dispense 80 µL of **Solution #1** into each well of the microplate.
4. Place the plate on the carrier and run the **W-192_EVAC_TEST**. This protocol evacuates all of the wells, leaving a small amount of residual fluid.
5. When the program is completed, remove the plate and weigh it immediately because evaporation will affect the results. This is the **Total Residual Weight,** in grams.
6. Visually inspect the plate and note if any wells appear to have considerably more liquid in them than others.
7. Using the *Evacuation Efficiency Test Worksheet* on page 170, perform initial data reduction:
    a. Divide the Total Residual Weight by **384** to find the **Mean Residual Weight**.
    b. The Mean Residual Weight should be **<= 0.002 g**.

If the Mean Residual Weight is *greater than* 0.002 g, or if one or more wells appear to have much more liquid than the others, the washer failed the test.

If the test fails once, **Troubleshoot as follows:**

- If the problem appears to be related to particular wells, clean those aspiration tubes: run **AutoClean**, and/or remove the manifold and thoroughly clean the tubes with the stylus (see ***Chapter 5, Maintenance*** for instructions.) When finished, retry the test.
- Failure of this test is commonly caused by improper aspiration tube positioning, usually because a microplate other than the recommended Corning Costar® 384 was used. If you prefer to use a different plate, modify the **Aspirate Positioning**, i.e., Z-axis to fix the height, or X- or Y-axes parameters in a copy of the **W-192_EVAC_TEST** protocol. After making needed changes, retry the test using a new microplate.

  If you *are* using the Corning Costar 384 microplate, run **AutoClean** and/or remove the manifold and clean the aspiration tubes with the stylus. When finished, retry the test using a new microplate.

- If the test fails a second time: perform the diagnostic procedure below.

### *Evacuation Diagnostic Procedure (192)*

*Perform these steps if the Evacuation Efficiency Test (192) fails twice. This test will confirm which aspirate tube(s) may be clogged, or if the plate's alignment or position is the problem.*

1. If you have not already done so, repeat steps 2 through 7 of the previous test, using **Solution #2** for the dispense fluid. Be sure to recalculate the **Mean Residual Weight**.
2. Pipette or dispense 80 µL of **Solution #1** into each well, on top of the residual solution.
3. Shake the plate to achieve uniform distribution of the remaining dye in each well.
4. Read the plate in an optical reader (blank on air), using the dual-wavelength method (630 nm - 450 nm), then print or export the results.
5. Using the *Evacuation Diagnostic* worksheet on page 170, perform data reduction:
   a. Calculate the sum of the OD values for all 384 wells, then divide by **384** to determine the **Mean OD** for the plate.
   b. Divide the Mean OD by the **Mean Residual Weight** (from step 1) to find the **Residual Factor.**
   c. For each well, divide its OD value by the Residual Factor to find its **Residual Weight.**

      Each well's Residual Weight should be **<= 0.002 g.**

If one or more wells have a Residual Weight *greater than* 0.002 g, review the data to determine which well, or wells, is causing the problem.

- If the problem appears to be related to particular wells, clean the associated aspiration tubes: run **AUTOCLEAN** and/or remove the manifold and thoroughly clean the tubes with the stylus (all models). See ***Chapter 4, Preventive Maintenance*** for complete instructions. When finished, retry the test.
- If the problem appears to be related to a particular region, edge, or corner of the plate, review the alignment and flatness of the plate on the carrier.

- **Please do not adjust** the carrier adjustment screws! Contact your BioTek dealer if you suspect an alignment problem.

- For additional suggestions, refer to ***Chapter 6, Troubleshooting***.
- If the test continues to fail, contact BioTek Instruments.

## Annual Buffer Switching Module Test

This procedure tests all the Buffer valves.

- ❖ Perform this test during the **annual OQ** only.
- ❖ The **Dispense Precision** test must pass before the **annual** test for Valves A-D can be performed.

1. Empty the waste bottle now, and then as needed throughout this procedure.
2. Fill each of the supply bottles connected to **Buffer valves A**, **B**, **C**, and **D** with three liters (3000 mL) of deionized water. Place the bottles on a surface level with the EL406, i.e., the adjacent (lab bench) area.
3. Run the Maintenance protocol **W-PRIME_ALL_BFRS** two or three times to prime the fluid lines, manifold, and the valves.

***Repeat the following steps <u>for each valve</u>:***

4. Edit the applicable pre-defined protocols: **W-96_DISP_TEST** or **W-192_DISP_TEST** to use the Buffer valve currently being tested.
5. Place a new 96- or 384-well microplate on the balance and zero the balance.
6. Place the microplate on the carrier and run the Dispense protocol.
7. When the program is finished, carefully remove the plate and weigh it. This is the **Total Dispense Weight** in grams.
   - The Total Dispense Weight should be:
     - **28.8 grams ± 10%** (between 25.92 g and 31.68 g) for the 96-Tube test.
     - **30.72 grams, ± 10%** (between 27.65 g and 33.79 g) for 192-Tube test.
   - If the weight falls above this range, the valve may be defective. Contact BioTek.
   - If the weight falls below this range, the valve may be contaminated with fungi or proteins and must be cleaned using an appropriate enzyme, alcohol, or a diluted bleach solution, depending on the contaminant. See ***Removing Protein Residuals and Fungi Growth*** in the Maintenance section for suggestions. After cleaning the valve and tubing, retry the test. If the test continues to fail, contact BioTek.
8. Record the results in the applicable ***Dispense Precision Test Worksheet***.

## Evacuation Efficiency Test (128)

Begin this test by dispensing the test solution with Syringe A or B to a 1536-well plate using one of the pre-defined QC protocols provided:

| | | |
|---|---|---|
| SA-1536_DISP_TST | Dispense precision test protocol for 32-tube Syringe A manifold. | 20 |
| SB-1536_DISP_TST | Dispense precision test protocol for 32-tube Syringe B manifold. | 21 |

1. Fill the Syringe dispenser's supply bottle with 100 mL of Solution 1 (see page 156) and **PRIME** the tubing and manifold.
2. Place a new 1536-well microplate on the balance and zero the balance.
3. **RUN** the appropriate pre-defined protocol (listed above) to dispense 6 µL to 512 wells of the plate.
4. After performing SA-1536_DISP_TST or SB-1536_DISP_TST, run the **W-1536_EVAC_TEST**. This protocol evacuates all of the wells, leaving a small amount of residual fluid.
5. When the program is completed, remove the plate and weigh it immediately because evaporation will affect the results. This is the **Total Residual Weight,** in grams.
6. Visually inspect the plate and note if any wells appear to have considerably more liquid in them than others.
7. Using the *Evacuation Efficiency Test Worksheet* on page 172, perform initial data reduction:
    a. Divide the Total Residual Weight by **512** to find the **Mean Residual Weight**.
    b. The Mean Residual Weight should be **<= 0.0001 g**.

If the Mean Residual Weight is *greater than* 0.0001 g, or if one or more wells appear to have much more liquid than the others, the washer failed the test.

If the test fails, **Troubleshoot as follows:**

- If the problem appears to be related to particular wells, clean those aspiration tubes: run **AutoClean**, and/or remove the manifold and thoroughly clean the tubes with the stylus (see ***Chapter 5, Maintenance*** for instructions.) When finished, retry the test.
- Failure of this test is commonly caused by improper aspiration tube positioning, usually because a microplate other than the recommended Nunc® #264710 was used. If you prefer to use a different plate, modify the **Aspirate Positioning**, i.e., Z-axis to fix the height, or X- or Y-axes parameters in a copy of the **W-1536_EVAC_TEST** protocol. After making needed changes, retry the test using a new microplate.

If you *are* using the Nunc 1536-well microplate, run **AutoClean** and/or remove the manifold and clean the aspiration tubes with the stylus. When finished, retry the test using a new microplate.

- For additional suggestions, refer to ***Chapter 6, Troubleshooting***.
- If the test continues to fail, contact BioTek Instruments.

## Dispense Precision Test Worksheet

*96-Tube Manifold*

| | |
|---|---|
| **Serial Number:** | |

| **Calculations** | |
|---|---|
| Standard Deviation:<br>(calculate using spreadsheet program) | |
| Mean OD:<br>(sum of all wells ÷ number of wells) | |
| % Coefficient of Variation:<br>((Standard Deviation ÷ Mean OD) x 100) | |
| % CV <= 3.0? | ❑ Pass ❑ Fail |

| | |
|---|---|
| **Date:** | |
| **Test Performed By:** | |

# Evacuation Efficiency Test Worksheet

*96-Tube Manifold*

| **Serial Number:** | |
|---|---|

| **Standard Test** | |
|---|---|
| Total Residual Weight: | g |
| Verification that wells are consistent in appearance: | ❑ Pass ❑ Fail |
| Mean Residual Weight (Total Residual Weight ÷ 96): | g |
| Mean Residual Weight <= 0.002 g? | ❑ Pass ❑ Fail |

| **Evacuation Diagnostics Test (check here ❑ if not performed)** | |
|---|---|
| Mean OD for the plate (Sum of all wells ÷ 96): | |
| Residual Factor (Mean OD ÷ Mean Residual Weight): | |
| *Calculate the Residual Weight for each well: well OD ÷ Residual Factor* | |
| Every Residual Weight per well <= 0.002 g? | ❑ Yes ❑ No |

| **Date:** | |
|---|---|
| **Test Performed By:** | |

# Dispense Precision Test Worksheet

*96-Tube Manifold*
*Annual Buffer Switching Test*

| | |
|---|---|
| **Serial Number:** | |

| **Calculations for Valve A** | |
|---|---|
| Standard Deviation: | |
| Mean OD (Sum of all wells ÷ Number of wells): | |
| % CV (Standard Deviation ÷ Mean OD x 100): | |
| % CV <= 3.0? | ❑ Pass ❑ Fail |

| **Calculations for Valves A-D (Annual OQ only, check here ❑ if not performed)** | | |
|---|---|---|
| | **Total Dispense Weight** | **28.8 g ±10%? (25.92 g - 31.68 g)** |
| **Valve A** | grams | ❑ Pass ❑ Fail |
| **Valve B** | grams | ❑ Pass ❑ Fail |
| **Valve C** | grams | ❑ Pass ❑ Fail |
| **Valve D** | grams | ❑ Pass ❑ Fail |

| | |
|---|---|
| **Date:** | |
| **Test Performed By:** | |

# Dispense Precision Test Worksheet

*192-Tube Manifold*

| | |
|---|---|
| **Serial Number:** | |

| **Calculations** | |
|---|---|
| Standard Deviation:<br>(calculate using spreadsheet program) | |
| Mean OD:<br>(sum of all wells ÷ number of wells) | |
| % Coefficient of Variation:<br>((Standard Deviation ÷ Mean OD) x 100) | |
| % CV <= 4.0? | ❑ Pass ❑ Fail |

| | |
|---|---|
| **Date:** | |
| **Test Performed By:** | |

# Evacuation Efficiency Test Worksheet

*192-Tube Manifold*

| **Serial Number:** | |
|---|---|

| **Standard Test** | |
|---|---|
| Total Residual Weight: | g |
| Verification that wells are consistent in appearance: | ❑ Pass ❑ Fail |
| Mean Residual Weight (Total Residual Weight ÷ 384): | g |
| Mean Residual Weight <= 0.002 g? | ❑ Pass ❑ Fail |

| **Evacuation Diagnostics Test (check here ❑ if not performed)** | |
|---|---|
| Mean OD for the plate (sum of all wells ÷ 384): | |
| Residual Factor (Mean OD ÷ Mean Residual Weight): | |
| *Calculate the Residual Weight for each well: well OD ÷ Residual Factor* | |
| Every Residual Weight per well <= 0.002 g? | ❑ Yes ❑ No |

| **Date:** | |
|---|---|
| **Test Performed By:** | |

# Dispense Precision Test Worksheet

*192-Tube Manifold*
*Annual Buffer Switching Test*

| | |
|---|---|
| **Serial Number:** | |

| **Calculations for Valve A** | |
|---|---|
| Standard Deviation: | |
| Mean OD (Sum of all wells ÷ Number of wells): | |
| % CV (Standard Deviation ÷ Mean OD x 100): | |
| % CV <= 4.0? | ❑ Pass ❑ Fail |

| **Calculations for Valves A-D (Annual OQ only, check here ❑ if not performed)** | | |
|---|---|---|
| | **Total Dispense Weight** | **30.72 g ± 10%? (27.65 g – 33.79 g)** |
| **Valve A** | grams | ❑ Pass ❑ Fail |
| **Valve B** | grams | ❑ Pass ❑ Fail |
| **Valve C** | grams | ❑ Pass ❑ Fail |
| **Valve D** | grams | ❑ Pass ❑ Fail |

| | |
|---|---|
| **Date:** | |
| **Test Performed By:** | |

## Evacuation Efficiency Test Worksheet

*128-Tube Aspirate Manifold*

| | |
|---|---|
| **Serial Number:** | |

| **Standard Test** | |
|---|---|
| Total Residual Weight: | g |
| Verification that wells are consistent in appearance: | ❑ Pass ❑ Fail |
| Mean Residual Weight (Total Residual Weight ÷ 512): | g |
| Mean Residual Weight <= 0.0001 g? | ❑ Pass ❑ Fail |

| | |
|---|---|
| **Date:** | |
| **Test Performed By:** | |

# Peri-pump Dispense Precision and Accuracy Tests

### Dispense Precision and Accuracy Specifications

| Cassette | Precision | Accuracy |
|---|---|---|
| **1µL** | < 10%CV @ 1 µL per well | ± 10% @ 1 µL per well |
| | < 5%CV @ 2 µL per well* | ± 5% @ 2 µL per well* |
| **1µL 1536** | < 10%CV @ 1 µL per well | ± 10% @ 1 µL per well |
| | < 5%CV @ 2 µL per well* | ± 5% @ 2 µL per well* |
| **5µL** | < 5%CV @ 5 µL per well | ± 4% @ 5 µL per well |
| | < 2.5%CV @ 10 µL per well* | ± 2% @ 10 µL per well* |
| **10µL** | < 4%CV @ 10 µL per well | ± 4% @ 10 µL per well |
| | < 2%CV @ 20 µL per well* | ± 2% @ 20 µL per well* |

- * These specifications are for these dispense volumes and higher.
- Note: The above specifications are for typical performance. For IQ/PQ/OQ purposes, add 1.0% additional tolerance to the Precision %CV to accommodate various test solutions, off-peak wavelengths, reader errors, and pipette errors.

### Liquid Testing Methodology

Tare an empty plate on a balance. Use the Peri-pump to dispense a quantity of fluid with a known dye concentration to the wells. Weigh the plate to obtain the weight of the fluid dispensed. Pipette deionized water on top of the dye to bring the wells up to a more optically measureable volume. Read the wells in a microplate reader and determine the percentage Coefficient of Variance (%CV) among all wells, and the accuracy of the volume dispensed in each well (% Accuracy Error).

BioTek recommends performing two tests, one at the volume that matches the cassette type and another that best represents the cassette type and dispense volume most common to your applications:

| Tests – Solutions | Cassette Types | | | |
|---|---|---|---|---|
| | 1 µL | 1 µL 1536 | 5 µL | 10 µL |
| 1 µL | √ | √ | | |
| 5 µL | | | √ | |
| 10 µL | √ | √ | | √ |
| 50 µL | | | √ | |

| Tests – Solutions | Cassette Types | | | |
|---|---|---|---|---|
| | 1 µL | 1 µL 1536 | 5 µL | 10 µL |
| 100 µL | | | | √ |
| 1536 µL | | √ | | |

❖ A single aliquot for a cassette type is the smallest volume unit recommended for it. 1 µL for the 1 µL cassette, 5 µL for the 5 µL cassette, and 10 µL for the 10 µL cassette.

**1 µL Test:**

- Confirms performance of 1 µL cassettes when dispensing a single aliquot (1/4 turn of pump) into each well of the plate.
- Dispenses 1 µL into each well using the **1 µL Solution**, and requires an additional 150 µL of DI water to raise the fluid level for optimal reading.

**5 µL Test:**

- Confirms performance of 5 µL cassettes when dispensing a single aliquot (1/4 turn of pump) into each well of the plate.
- Dispenses 5 µL into each well using the **5 µL Solution**, and requires an additional 150 µL of DI water to raise the fluid level for optimal reading.

**10 µL Test:**

- Confirms performance of 1 µL cassettes when dispensing 10 aliquots (2 1/2 turns of pump) and 10 µL cassettes when dispensing a single aliquot (1/4 turn of the pump) into each well of the plate.
- Dispenses 10 µL into each well using the **10 µL Solution**, and requires an additional 100 µL of DI water to raise the fluid level for optimal reading.

**50 µL Test:**

- Confirms performance of 5 µL cassettes when dispensing 10 aliquots (2 1/2 turns of pump) into each well of the plate.
- Dispenses 50 µL into each well using the **50 µL solution**, and requires an additional 100 µL of DI water to raise the fluid level for optimal reading.

**100 µL Test:**

- Confirms performance of 10 µL cassettes when dispensing 10 aliquots (2 1/2 turns of pump) into each well of the plate.
- Dispenses 100 µL into each well using the **100 µL solution**, and requires an additional 50 µL of DI water to raise the fluid level for optimal reading.

**1536 Test:**

- Confirms the alignment of the tips; that the cassette is firing straight into the wells.
- Dispenses 6µL into columns 2, 4, 19-30, 45, 47 of a 1536 well plate using **1536 solution**. Then, requires performing the **1 µL Test** described above.

## Materials

- Corning® Costar #3590 96-well plates, or equivalent
- 1536-well plates, Nunc #264710
- Precision balance with readability of 0.0001 g resolution is preferable, 0.001 g resolution is acceptable, and capacity of 100 g minimum
- Pipettes and graduated beakers
- Microplate absorbance reader capable of dual wavelength reading at 630 and 450 (or 405) nm
- BioTek blue dye solution, PN 7773001, or equivalent to create the test solutions described on the following page. See also the important notes on page 149.

## Test Solutions

Unique concentrations of the test fluid are described here, each one corresponds to a specific dispense volume. Prepare the solutions you will need to validate the cassette types and dispense volumes used most commonly in your applications.

❖ The 5 µL Solution is used to make the higher volume test solutions.

| | |
|---|---|
| **1 µL Solution** | Using BioTek's 10X concentrated blue dye solution (PN 7773001), mix 5 mL of DI water with 8 mL of the blue dye solution. |
| **5 µL Solution** | Using BioTek's 10X concentrated blue dye solution (PN 7773001), mix 100 mL of DI water with 10 mL of the blue dye solution. |
| **10 µL Solution** | Mix 25 mL of DI water with 20 mL of the 5 µL Solution. |
| **50 µL Solution** | Mix 45 mL of DI water with 5 mL of the 5 µL Solution. |
| **100 µL Solution** | Mix 40 mL of DI water with 2 mL of the 5 µL Solution. |
| **1536 Solution** | Mix 5 mL of 70% Isopropyl Alcohol with 3 mL of the 5 µL Solution and 35 mL of DI H2O. |

## Performing the Precision and Accuracy Tests

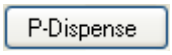
**Prerequisite:**

- Prepare the test solutions as described above.
- Make a copy of the applicable worksheet beginning on page 178.

## Procedure:

1. Install the cassette to be tested.
2. Turn on the EL406 and make sure the cassette (CASS) type setting is correct.
3. Turn on the balance.
4. Fill a beaker or other vessel with the test solution.
5. Define a **protocol** and save it for reuse. Set the parameters based on the desired test volume:

❖ A pre-defined protocol for the 1536 Test is shipped with the instrument and installed on your PC when you install the LHC: P-1536_DISP_TEST, run number 22 on the keypad.

- 

Add a Peri-pump **DISP**ense step to the protocol.
- Set the **Dispense Volume** to match the Test:

| Test | Volume |
|---|---|
| 1 µL | 1 µL |
| 5 µL | 5 µL |
| 10 µL | 10 µL |
| 50 µL | 50 µL |
| 100 µL | 100 µL |
| 1536 | 6 µL |

- Set the **Flow Rate** to:
  - 1 µL cassette = Medium
  - 5 µL cassette = High
  - 10 µL cassette = High
- Optionally, choose to Require the specific cassette type under test.
- Define a **Pre-dispense**: set the volume to **10** µL and the Number of Pre-dispenses (cycles) to **2**.

6. Place a clean/new microplate on the balance and tare the balance.
7. Put the Tube Organizer into the test fluid vessel and **Prime** the tubing until any large air bubbles are removed.
8. **Run** the dispense protocol.

9. Place the plate on the balance and record the **Total Dispense Weight** in the worksheet.
10. Using a calibrated hand pipette, add the specified amount of deionized water to each well to raise the fluid level to approximately 150 µL.

| Test | Volume |
|---|---|
| 1 µL | 150 µL |
| 5 µL | 150 µL |
| 10 µL | 100 µL |
| 50 µL | 100 µL |
| 100 µL | 50 µL |
| 1536 | 0 µL |

11. Read the plate in an absorbance reader using the dual-wavelength method: read the plate at 630 nm and 450 nm.
12. Calculate the **Delta OD**: (630 nm – 450 nm), **Mean Absorbance**, **Standard Deviation**, and the **%CV** for the wells under test. **%CV** = (Standard Deviation ÷ Mean) * 100.
13. Print the report, obtain required signatures, and store it according to regulatory guidelines.

If one or more of your tests are failing, make sure the dispense tubes are not clogged, (follow the Unclog the Dispense Tips instructions in the ***Maintenance*** chapter). If that doesn't work, recalibrate the cassette and repeat the test(s). If your tests continue to fail, contact BioTek's Technical Assistance Center (TAC).

## Documenting Test Results

The following pages contain Dispense Precision & Accuracy Test Worksheets. We recommend you make copies of the appropriate pages and use them to record your calculations and test results.

Alternatively, you can purchase the instrument qualification package, which contains additional tools for conducting test procedures and recording the results, including logbooks and Excel® spreadsheets.

## EL406 Peri-pump Dispense Precision & Accuracy Test Worksheet
## 1 µL Cassette Test

| 1 µL Dispense Precision Test | |
|---|---|
| Standard Deviation *(SD)*: | |
| Mean Absorbance *(sum of all wells ÷ 96)* | |
| % CV *(SD ÷ Mean x 100)* | % |
| % CV must be < 11.0% | ❑ Pass ❑ Fail |

| 1 µL Dispense Accuracy Test | |
|---|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight: *(mL/well x number of wells dispensed)* | grams |
| % Accuracy Error: *(Actual Weight – Expected Weight) ÷ Expected Weight x 100* | % |
| % Accuracy Error must be < 10.0% | ❑ Pass ❑ Fail |
| Visual verification that no well varies considerably from the others | ❑ Pass ❑ Fail |

| | |
|---|---|
| **Cassette Serial Number:** | |
| **Tests Performed By:** | |
| **Date:** | |
| **Reviewed / Approved By:** | |
| **Date:** | |

# EL406 Peri-pump Dispense Precision & Accuracy Test Worksheet
# 5 µL Cassette Test

| **5 µL Dispense Precision Test** | |
|---|---|
| Standard Deviation *(SD)*: | |
| Mean Absorbance *(sum of all wells ÷ 96)* | |
| % CV *(SD ÷ Mean x 100)* | % |
| % CV must be < 6.0% | ❑ Pass ❑ Fail |

| **5 µL Dispense Accuracy Test** | |
|---|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight:<br>*(mL/well x number of wells dispensed)* | grams |
| % Accuracy Error:<br>*(Actual Weight – Expected Weight) ÷ Expected Weight x 100* | % |
| % Accuracy Error must be < 4.0% | ❑ Pass ❑ Fail |
| Visual verification that no well varies considerably from the others | ❑ Pass ❑ Fail |

| | |
|---|---|
| **Cassette Serial Number:** | |
| **Tests Performed By:** | |
| **Date:** | |
| **Reviewed / Approved By:** | |
| **Date:** | |

# EL406 Peri-pump Dispense Precision & Accuracy Test Worksheet
# 1 µL Cassette Test

| **10 µL Dispense Precision Test** | |
|---|---|
| Standard Deviation *(SD)*: | |
| Mean Absorbance *(sum of all wells ÷ 96)* | |
| % CV *(SD ÷ Mean x 100)* | % |
| % CV must be < 6.0% | ❑ Pass ❑ Fail |

| **10 µL Dispense Accuracy Test** | |
|---|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight:<br>*(mL/well x number of wells dispensed)* | grams |
| % Accuracy Error:<br>*(Actual Weight – Expected Weight) ÷ Expected Weight x 100* | % |
| % Accuracy Error must be < 5.0% | ❑ Pass ❑ Fail |
| Visual verification that no well varies considerably from the others | ❑ Pass ❑ Fail |

| | |
|---|---|
| **Cassette Serial Number:** | |
| **Tests Performed By:** | |
| **Date:** | |
| **Reviewed / Approved By:** | |
| **Date:** | |

# EL406 Peri-pump Dispense Precision & Accuracy Test Worksheet
# 10 µL Cassette Test

| **10 µL Dispense Precision Test** | |
|---|---|
| Standard Deviation *(SD)*: | |
| Mean Absorbance *(sum of all wells ÷ 96)* | |
| % CV *(SD ÷ Mean x 100)* | % |
| % CV must be < 5.0% | ❑ Pass ❑ Fail |

| **10 µL Dispense Accuracy Test** | |
|---|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight:<br>*(mL/well x number of wells dispensed)* | grams |
| % Accuracy Error:<br>*(Actual Weight – Expected Weight) ÷ Expected Weight x 100* | % |
| % Accuracy Error must be < 4.0% | ❑ Pass ❑ Fail |
| Visual verification that no well varies considerably from the others | ❑ Pass ❑ Fail |

| | |
|---|---|
| **Cassette Serial Number:** | |
| **Tests Performed By:** | |
| **Date:** | |
| **Reviewed / Approved By:** | |
| **Date:** | |

# EL406 Peri-pump Dispense Precision & Accuracy Test Worksheet
## 5 µL Cassette Test

| **50 µL Dispense Precision Test** | |
|---|---|
| Standard Deviation *(SD)*: | |
| Mean Absorbance *(sum of all wells ÷ 96)* | |
| % CV *(SD ÷ Mean x 100)* | % |
| % CV must be < 3.5% | ❑ Pass ❑ Fail |

| **50 µL Dispense Accuracy Test** | |
|---|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight:<br>*(mL/well x number of wells dispensed)* | grams |
| % Accuracy Error:<br>*(Actual Weight – Expected Weight) ÷ Expected Weight x 100* | % |
| % Accuracy Error must be < 2.0% | ❑ Pass ❑ Fail |
| Visual verification that no well varies considerably from the others | ❑ Pass ❑ Fail |

| | |
|---|---|
| **Cassette Serial Number:** | |
| **Tests Performed By:** | |
| **Date:** | |
| **Reviewed / Approved By:** | |
| **Date:** | |

# EL406 Peri-pump Dispense Precision & Accuracy Test Worksheet
# 10 µL Cassette Test

| **100 µL Dispense Precision Test** | |
|---|---|
| Standard Deviation *(SD)*: | |
| Mean Absorbance *(sum of all wells ÷ 96)* | |
| % CV *(SD ÷ Mean x 100)* | % |
| % CV must be < 3.0% | ❑ Pass ❑ Fail |

| **100 µL Dispense Accuracy Test** | |
|---|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight:<br>*(mL/well x number of wells dispensed)* | grams |
| % Accuracy Error:<br>*(Actual Weight – Expected Weight) ÷ Expected Weight x 100* | % |
| % Accuracy Error must be < 2.0% | ❑ Pass ❑ Fail |
| Visual verification that no well varies considerably from the others | ❑ Pass ❑ Fail |

| | |
|---|---|
| **Cassette Serial Number:** | |
| **Tests Performed By:** | |
| **Date:** | |
| **Reviewed / Approved By:** | |
| **Date:** | |

# EL406 Peri-pump Dispense Precision & Accuracy Test Worksheet
# 1536 Cassette Test

| 1536 Dispense Precision Test | |
|---|---|
| Standard Deviation *(SD)*: | |
| Mean Absorbance *(sum of all wells ÷ 96)* | |
| % CV *(SD ÷ Mean x 100)* | % |
| % CV must be < 6.0% | ❑ Pass ❑ Fail |

| 1536 Dispense Accuracy Test | |
|---|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight: *(mL/well x number of wells dispensed)* | grams |
| % Accuracy Error: *(Actual Weight – Expected Weight) ÷ Expected Weight x 100* | % |
| % Accuracy Error must be < 5.0% | ❑ Pass ❑ Fail |
| Visual verification that no well varies considerably from the others | ❑ Pass ❑ Fail |

| | |
|---|---|
| **Cassette Serial Number:** | |
| **Tests Performed By:** | |
| **Date:** | |
| **Reviewed/Approved By:** | |
| **Date:** | |

# Syringe Dispenser Precision and Accuracy Tests

## Dispense Precision and Accuracy Specifications

❖ **Note:** The specifications listed here are for typical performance. For IQ/PQ/OQ testing purposes, add 1.0% additional tolerance to the Precision **%CV** to accommodate various test solutions, off-peak wavelengths, reader errors, and pipette errors.

| | Precision | Accuracy |
|---|---|---|
| **384-well dispense** | < 3 %CV @ 80 µL/well | ± 1.25% @ 80 µL/well |
| | < 6 %CV @ 20 µL/well | ± 5% @ 20 µL/well |
| **96-well dispense** | < 3 %CV @ 160 µL/well | ± 1.25% @ 160 µL/well |
| | < 6 %CV @ 40 µL/well | ± 5% @ 40 µL/well |
| **1536-well dispense** | < 12% CV @ 6 µL/ well | ± 5% @ 6 µL/well |

## Materials

- Plates: 384-well plates for testing the 16-tube dispensers (which can be replaced with 96-well plates if more applicable for your lab); 1536-well plates for testing the 32-tube dispensers.
- Precision balance with capacity of 100 g minimum and readability of 0.01 g resolution
- Pipettes and graduated beakers
- Microplate absorbance reader capable of dual wavelength reading at 630 and 405 (or 450) nm
- The test solutions described below. See also the ***Important Recommendations for All Liquid Tests*** on page 149.

## Test Solutions

| | |
|---|---|
| **20 µL Solution** | Mix 10 mL of BioTek's blue dye solution with 100 mL of deionized water to create a dilution of the concentrate.<br>Mix 160 mL of deionized water with 20 mL of the diluted concentrate. |
| **80 µL Solution** | Mix 120 mL of deionized water with 40 mL of the **20 µL Solution**. |
| **1536 Solution** | Mix 21 mL of 70% Isopropyl Alcohol with 13 mL of the **20 µL Solution** and 150 mL of DI H2O. |

Two pre-defined QC protocols are provided for qualifying the 32-tube dispensers:

| | | |
|---|---|---|
| SA-1536_DIS_TEST | Dispense precision test protocol for 32-tube Syringe A manifold. | 20 |
| SB-1536_DIS_TEST | Dispense precision test protocol for 32-tube Syringe B manifold. | 21 |

## Perform the Syringe Dispense Precision & Accuracy Test

Perform two tests for each syringe: use two 384-well plates (unless only 96-well plates are used in your lab) with two dye solutions.

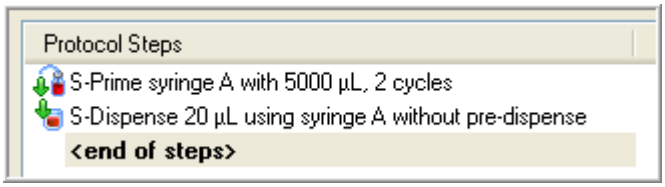
1. Prepare the syringe dispenser to be tested:

   **Tests 1:** Use the 20 µl solution

   **Tests 2:** Use the 80 µl solution

   **Tests 3:** Use the 1536 µL solution

2. Create and save protocols, two for each Syringe, for the tests as follows (except when testing the 32-tube dispensers, skip to step 3):




   1. Define a **Prime** step:
      - Volume = **5000 µL**
      - Flow Rate = **5**
      - Number of cycles = **2**
   2. Define a **Dispense** step for each test:

      **Test 1:** Plate Type **384**, Volume = **20 µL/well**; (Alternatively, Plate Type **96**, Volume = **40 µL/well**)

      **Test 2:** Plate Type **384**, Volume = **80 µL/well**; (Alternatively, Plate Type **96**, Volume = **160 µL/well**)

      Set Flow Rate to **2** for all tests.

3. Place a clean, empty 384-well or 1536-well (or 96-well) microplate on the balance and tare the balance.
4. Place the microplate on the carrier and run the protocol created in step 2 or shipped with the instrument:

| | | |
|---|---|---|
| SA-1536_DIS_TEST | Test protocol for 32-tube Syringe A. | 20 |

| | | |
|---|---|---|
| SB-1536_DIS_TEST | Test protocol for 32-tube Syringe B. | 21 |

**Important**: Fully prime the Syringe tubing to remove any air bubbles before running these pre-defined protocols.

5. Place the plate on the balance and record the **Total Dispense Weight**. This value will be used to calculate the % Accuracy Error in step 10.
6. **For Test 1 Only**: Use a calibrated hand pipette to dispense deionized water on top of the dye solution in the wells.

   **384-well:** Pipette 60 µL/well (resulting in 80 µL/well)

   **96-well:** Pipette 120 µL/well (resulting in 160 µL/well)

   Shake the plate on an orbital shaker or in a microplate reader for 15 seconds, or lightly tap the side of the plate with your finger to agitate the contents of the wells.
7. Read the plate in an absorbance reader using the dual-wavelength method, to reduce the influence of scratches and foreign particles that could be in the well. See the recommended wavelengths on page 149. Print or export the results.

❖ For steps 8 and 9, enter the results in copies of the test Worksheets beginning on page 189.

8. Calculate and report the Mean absorbance, Standard Deviation, and the %CV for the wells under test. **%CV** = (Standard Deviation ÷ Mean) * 100.
9. The **% Accuracy Error** calculation is:

   (Actual Weight – Expected Weight) ÷ Expected Weight x 100

   Subtract the expected dispense weight (see below) from the Actual (Total) Dispense Weight (from step 6), and divide the result by the expected weight. Multiply the result by 100.

   The **Expected Dispense Weight** is the volume dispensed per well in mL multiplied by the number of wells dispensed. For example, if 40 µL is dispensed to 96 wells, the expected weight is 0.040 x 96 = 3.84 grams. We have calculated some expected dispense weights for you:

| Test | # of wells | Volume | Expected Weight |
|---|---|---|---|
| **Test 1:** | 384 wells | 20 µL/well | 7.68 grams |
| | 96 wells | 40 µL/well | 3.84 grams |
| **Test 2:** | 384 wells, | 80 µL/well | 30.72 grams |
| | 96 wells | 160 µL/well | 15.36 grams |
| **Test 3:** | 512 wells (of a 1536-well plate) | 6 µL/well | 3.024 grams |

10. Analyze your test results. The following is the Pass criteria for each test:

| | **%CV** | **% Accuracy Error** |
|---|---|---|
| **Test 1:** | < 6.0%* | ± 5.0% |
| **Test 2:** | < 3.0%* | ± 1.25% |
| **Test 3:** | < 12.0 % | ± 5.0% |

* The specifications listed on page 185 are for *typical* performance. For IQ/PQ/OQ purposes, we have added 1.0% additional tolerance to the **%CV** to accommodate various test solutions, off-peak wavelengths, reader errors, and pipette errors.

If one or more of your tests are failing, clean the dispense tubes with the stylus, re-prime the manifold, and repeat the test(s). If your tests continue to fail, contact BioTek's Technical Assistance Center.

## Documenting Test Results

The following pages contain Dispense Precision & Accuracy Test Worksheets. We recommend you make copies of the appropriate pages and use them to record your calculations and test results.

Each Worksheet records calculations and pass/fail test results for an individual test.

# EL406 Syringe Dispenser Precision & Accuracy Test Worksheet
## Test 1 / 384-Well Microplate / 20 µL Dispense

| **20 µL Dispense Precision Test** | |
|---|---|
| Standard Deviation *(SD)*: | |
| Mean Absorbance *(sum of all wells ÷ 384)* | |
| % CV *(SD ÷ Mean x 100)* | % |
| % CV must be < 6.0% | ❑ Pass ❑ Fail |

| **20 µL Dispense Accuracy Test** | |
|---|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight:<br>*(mL/well x number of wells dispensed)* | grams |
| % Accuracy Error:<br>*(Actual Weight – Expected Weight) ÷ Expected Weight x 100* | % |
| % Accuracy Error must be < 5.0% | ❑ Pass ❑ Fail |
| Visual verification that no well varies considerably from the others | ❑ Pass ❑ Fail |

| | |
|---|---|
| **Serial Number:** | |
| **Tests Performed By:** | |
| **Date:** | |
| **Reviewed / Approved By:** | |
| **Date:** | |

# EL406 Syringe Dispenser Precision & Accuracy Test Worksheet
## Test 2 / 384-Well Microplate / 80 µL Dispense

| 80 µL Dispense Precision Test | |
|---|---|
| Standard Deviation *(SD)*: | |
| Mean Absorbance *(sum of all wells ÷ 384)* | |
| % CV *(SD ÷ Mean x 100)* | % |
| % CV must be ≤ 3.0% | ❑ Pass ❑ Fail |

| 80 µL Dispense Accuracy Test | |
|---|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight:<br>*(mL/well x number of wells dispensed)* | grams |
| % Accuracy Error:<br>*(Actual Weight – Expected Weight) ÷ Expected Weight x 100* | % |
| % Accuracy Error must be < 1.25% | ❑ Pass ❑ Fail |
| Visual verification that no well varies considerably from the others | ❑ Pass ❑ Fail |

| | |
|---|---|
| **Serial Number:** | |
| **Tests Performed By:** | |
| **Date:** | |
| **Reviewed / Approved By:** | |
| **Date:** | |

# EL406 Syringe Dispenser Precision & Accuracy Test Worksheet
## Test 1 / 96-Well Microplate / 40 µL Dispense

| **40 µL Dispense Precision Test** | |
|---|---|
| Standard Deviation *(SD)*: | |
| Mean Absorbance *(sum of all wells ÷ 96)* | |
| % CV *(SD ÷ Mean x 100)* | % |
| % CV must be < 6.0% | ❑ Pass ❑ Fail |

| **40 µL Dispense Accuracy Test** | |
|---|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight:<br>*(mL/well x number of wells dispensed)* | grams |
| % Accuracy Error:<br>*(Actual Weight – Expected Weight) ÷ Expected Weight x 100* | % |
| % Accuracy Error must be < 5.0% | ❑ Pass ❑ Fail |
| Visual verification that no well varies considerably from the others | ❑ Pass ❑ Fail |

| | |
|---|---|
| **Serial Number:** | |
| **Tests Performed By:** | |
| **Date:** | |
| **Reviewed / Approved By:** | |
| **Date:** | |

# EL406 Syringe Dispenser Precision & Accuracy Test Worksheet
## Test 2 / 96-Well Microplate / 160 µL Dispense

| 160 µl Dispense Precision Test | |
|---|---|
| Standard Deviation *(SD)*: | |
| Mean Absorbance *(sum of all wells ÷ 96)* | |
| % CV *(SD ÷ Mean x 100)* | % |
| % CV must be ≤ 3.0% | ❑ Pass ❑ Fail |

| 160 µl Dispense Accuracy Test | |
|---|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight: *(ml/well x number of wells dispensed)* | grams |
| % Accuracy Error: *(Actual Weight – Expected Weight) ÷ Expected Weight x 100* | % |
| % Accuracy Error must be < 1.25% | ❑ Pass ❑ Fail |
| Visual verification that no well varies considerably from the others | ❑ Pass ❑ Fail |

| | |
|---|---|
| **Serial Number:** | |
| **Tests Performed By:** | |
| **Date:** | |
| **Reviewed / Approved By:** | |
| **Date:** | |

# EL406 Syringe Dispenser Precision & Accuracy Test Worksheet
## Test 3 / 1536-Well Microplate / 6 µL Dispense

| **6 µL Dispense Precision Test** | |
|---|---|
| Standard Deviation *(SD)*: | |
| Mean Absorbance *(sum of all wells ÷ 512)* | |
| % CV *(SD ÷ Mean x 100)* | % |
| % CV must be < 12.0% | ❑ Pass ❑ Fail |

| **6 µL Dispense Accuracy Test** | |
|---|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight:<br>*(mL/well x number of wells dispensed)* | grams |
| % Accuracy Error:<br>*(Actual Weight – Expected Weight) ÷ Expected Weight x 100* | % |
| % Accuracy Error must be < 5.0% | ❑ Pass ❑ Fail |
| Visual verification that no well varies considerably from the others | ❑ Pass ❑ Fail |

| | |
|---|---|
| **Serial Number:** | |
| **Tests Performed By:** | |
| **Date:** | |
| **Reviewed / Approved By:** | |
| **Date:** | |

Chapter 6

# Troubleshooting

This section contains guidelines for error recovery and troubleshooting performance problems.

# Error Recovery

**First Response**: Run a System Test (restart) to give the instrument an opportunity to restore its initial settings and computer communication capability.

> ❖ Generally an error tone from the instrument will announce an error condition and an error message will be displayed on-screen. The tone will cease when you respond to the message. Sometimes this requires pressing **Enter** on the keypad.

**Reboot your Computer and Instrument**: When you cannot run a system test, e.g., LHC is not responding, or when running a system test doesn't resolve the issue, turn off your computer and EL406, check all the cabling, i.e., make sure your serial or USB cable is in good condition and is properly connected to the PC and instrument, and then, power them on. This should refresh the devices and reset communication parameters.

### Error Codes

Most error conditions generate an error message that is displayed on the computer screen or keypad. The most common errors for new EL406 users are easily fixed:

| | |
|---|---|
| **6045** | Serial write error |

A "serial write" error is easily fixed by correcting the COM port setting defined in the protocol. Be sure to customize the Pre-defined Protocols when running them with the LHC to avoid this error in future.

| | |
|---|---|
| **810D** | To communicate, instrument must be at main menu. |

The 810D message appears when the instrument is busy, for example when AutoPrime is running. The LHC can only talk to the instrument when its main menu is displayed. Press the **Stop** button on the keypad, if desired, to end the current process and return to the main menu.

| | |
|---|---|
| **306** | Peri-pump pump cover is open. Close the pump cover and re-run protocol. |

To run the Peri-pump, its pump cover must be closed, protecting both the pump and the operator.

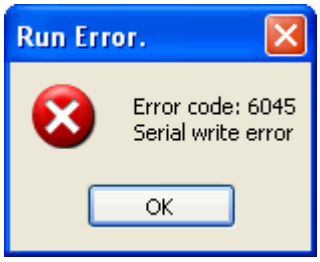
| | |
|---|---|
| **401** | Carrier Y motor failed positional verify |

If the plate carrier is not installed correctly, this instrument error will be displayed. Make sure the back right corner of the carrier is correctly seated in the little black knob attached to the transport rail. A slit on the bottom of the carrier allows it to fit into place.

See also: ***Appendix A: Error Codes***

# Communication Errors: EL406 to Computer

Here are some guidelines for troubleshooting communication errors.




A potentially common error, especially when using the Pre-defined Protocols, a "serial write" error, is easily fixed by correcting the COM port setting.

### Safety first

- To prevent damage to the instrument, always turn OFF the EL406 or the computer before removing or inserting a communications (serial or USB) cable.

### When the computer (PC) won't communicate with the EL406

1. **Confirm that the instrument passes its system self-test**. All BioTek instruments perform a self-test when turned on. The EL406 will not communicate if it fails an internal system test. An error message will be displayed when a test fails. Refer to the ***Operator's Manual*** to resolve the failure or contact BioTek TAC.
2. **Make sure the serial or USB cable is in perfect condition and properly attached** to the port defined in the Instrument Settings dialog (e.g. COM 1). Review the LHC Help Topic "About COM Ports" to learn about virtual COM ports when using a USB cable. Correct and reboot both PC and instrument. Test communication.
3. **Confirm that the serial/USB cable was obtained from BioTek**. Serial/USB cables are not universal. Contact BioTek customer service to purchase a factory-tested cable. After installing a known, good cable, reboot both PC and instrument. Test communication.
4. **Confirm** with your computer supplier or a local PC technician that **the serial port has been enabled**. For example, the IBM Thinkpad® was originally shipped with the serial port disabled. Correct and reboot both PC and EL406. Test communication.

# Troubleshooting

Potential problems and solutions are listed by device: Washer, Peri-pump on page 204, and Syringe dispenser on page 205.

## Washer Problems

| START-UP | | |
|---|---|---|
| ***Problem*** | ***Possible Cause*** | ***What To Do*** |
| Keypad display (LCD) not on. | Power cord not plugged in. | Check power connection. |
| Vacuum pump does not start, or shakes when turned on. | Vacuum pump is not turned on. | Flip the switch on the side of the vacuum pump to turn it on. |
| | Vacuum pump accessory cable not installed correctly. | Plug the vacuum pump accessory cable into the back of the instrument, **Accessory Outlet**. |
| | Too much residual vacuum force for pump. | Release the vacuum by loosening the waste bottle stopper. Reconnect and start again. |
| | Blown fuse in accessory outlet. | Plug the vacuum pump accessory cable into the back of the instrument, **Accessory Outlet**, not into a wall outlet. |
| | | Increase vacuum dissipation delay.<br>LHC: Tools>Instrument Utilities>Vacuum Dissipation Delay.<br>Keypad: Setup Menu>→ → VACDIS |
| | | Replace fuse (PN 46055) as described in ***Chapter 4***. |
| Repeated blown fuses. | Vacuum Dissipate Delay is set too low for the volume of the waste bottle. | See Solution above. If not enough time is allowed for the vacuum to dissipate, the pump will try to start while it is under a vacuum. The pump draws excessive current and blows the fuse. |
| | Pump has been flooded. | Remove the head from the pump and inspect it for corrosion, crystalline buildup or liquid. Contact BioTek TAC for information on pump rebuilding kits. |
| Carrier/manifold position error. | Manifold or carrier is being obstructed. | Remove obstruction. |
| | Motor, sensor, or electrical problem. | Turn instrument off, wait at least 15 seconds, and turn it back on. If the self-test does not pass, contact BioTek TAC. |
| | Misaligned carrier or manifold. | Contact BioTek TAC. |

| START-UP | | |
|---|---|---|
| ***Problem*** | ***Possible Cause*** | ***What To Do*** |
| | Incorrect manifold setting. | Make sure the washer manifold setting matches the installed manifold (96-tube or 192-tube).<br>LHC: Tools>Instrument Utilities>Washer>Manifold Selection.<br>Keypad: Setup Menu>WASH>MAN |

| FLUID ASPIRATION | | |
|---|---|---|
| ***Problem*** | ***Possible Cause*** | ***What To Do*** |
| Poor or uneven aspiration. | Insufficient or no vacuum. | Firmly seat the waste bottle covers. Ensure tubing is connected properly. Check all external tubing and in-line filter for kinks or clogs. If you are using an in-line vacuum filter, it may need to be replaced. |
| | | With the vacuum pump on, remove the vacuum pump tubing from the back of the instrument. Put your finger over the port; if there is no vacuum, contact BioTek TAC. |
| | Clogged aspiration tubes on the washer manifold. | Remove and clean the manifold as described in ***Chapter 4***. |
| | | Make sure the microplate carrier is level and the waste valve is not touching the bench. |
| | Aspirate height adjustment too high or too low. | Change the aspiration height (Z-axis position) in the protocol. |
| | Vacuum pump failure. | Contact BioTek TAC. |
| Uneven aspiration of water buffer. Some wells left full. | No surfactant in the buffer, such as Tween® 20. | Add surfactant to the buffer. If this is not possible, continue below. |
| | Insufficient vacuum. | BioTek offers a high-flow pump for assays using only water for the wash fluid. Contact BioTek for more information. |
| | Protocol settings not optimized. | Try these changes in this order and in combination, if necessary:<br>• Enable secondary aspiration.<br>• Increase aspiration Delay to 800 ms.<br>• Slow Travel Rate to 1 or 2. |
| | Aspiration tubes not properly positioned horizontally in wells. | If none of the tubes are bent, try adjusting the horizontal aspirate position (X-/Y-axis) in the protocol. |
| | Microplate not level in carrier, or strips not level in holder. | Reseat microplate in carrier or strips in holder. Make sure the carrier is clean.<br>Try a different microplate or strip holder. |

| FLUID ASPIRATION | | |
|---|---|---|
| ***Problem*** | ***Possible Cause*** | ***What To Do*** |
| | | If the problem is unresolved, the carrier may have to be realigned. Contact BioTek TAC. |
| Too much residual left in wells after aspiration. | Clogged vacuum filter. | If you are using an in-line vacuum filter (PN 49943), the filter may need to be cleaned or replaced. |
| | Waste bottle cover not properly sealed or fittings not properly connected. | Firmly seat the waste bottle stopper. Make sure tubing is connected properly. |
| | Manifold out of alignment or not moving freely. | Check for obstructions. If none are found, contact BioTek TAC. |
| | Protocol requires optimization. | To minimize the residuals, add a secondary (or crosswise) aspiration.<br>Other options include decreasing the Travel Rate or adding a Delay on the final aspiration. |
| | Aspirate tubes are bent. | Contact BioTek TAC. |

| FLUID DELIVERY | | |
|---|---|---|
| ***Problem*** | ***Possible Cause*** | ***What To Do*** |
| Unable to dispense fluid—units **without** the external Buffer Switching module. | Clogged fluid inlet filter. | Clean the fluid inlet filter as described in ***Chapter 4***. |
| | Supply tube inside the supply bottle is kinked or disconnected. | Straighten or connect supply tube. Make sure the end is cut; see Optimize Performance in ***Chapter 3***. |
| | Inlet tube not connected. | Make sure all tubing is connected properly. Check all external tubing for kinks or clogs. |
| | Clogged dispense tubes on the washer manifold. | Remove and clean the manifold as described in ***Chapter 4***. |
| | No wash or rinse fluid. | Fill bottles with appropriate fluid. Ensure bottles are clean and do not contain particles or organic material. |
| | System not primed. Large air pockets in tubing. | Run W-DAY_RINSE multiple times. |
| | Insufficient suction, clogged tubing, or faulty valve. | Perform Maintenance as described in ***Ch. 4***. If the problem persists, contact BioTek TAC. |
| Unable to dispense fluid—models **with** the external Buffer Switching module. | System not primed. Large air pockets in tubing. | Run W-DAY_RINSE multiple times. |
| | External valve module not connected to washer, or supply tubing set up incorrectly. | Check valve module cable and tubing. |

| FLUID DELIVERY | | |
|---|---|---|
| ***Problem*** | ***Possible Cause*** | ***What To Do*** |
| | Solenoid valve not opening. | Ensure module cable is plugged into the Valve Control port on the instrument's back panel. If plugged in, contact BioTek TAC. |
| Plate overfills (floods). | Dispense height too high. The aspirate tubes are too far above the microwells to prevent overflow. | Change the dispense height (Z-axis position) in the protocol. |
| | Dispense flow rate too low. | Define a higher dispense Flow Rate in the protocol. |
| | Cell wash flow rate 1 or 2 is used with 384-well plates. | Specify a non-CW dispense Flow Rate when using 384-well plates. |
| | Aspiration tubes hit bottom of trough during Prime or Maintenance. | Manifold may not be properly seated or mounted. Contact BioTek TAC. |
| | In-line vacuum filter plugged. | Replace or remove the in-line vacuum filter. |
| | Loose covers on waste bottles. | Firmly tighten waste bottle covers. |
| | Dispense rate too fast for volume selected. | Specify slower dispense Flow Rate or lower volume. |
| | Faulty vacuum pump. | Contact BioTek TAC. |
| | Insufficient or no vacuum. | Firmly seat the waste bottle covers.<br>Check all external tubing for kinks or clogs.<br>When the program begins, you should be able to hear the vacuum pump turn on. If it is not turning on, contact BioTek TAC.<br>If the vacuum pump turns on, remove the vacuum tubing from the back of the instrument and put your finger over the port. If there is no vacuum, contact BioTek TAC. |
| Uneven dispensing of fluid; wells not filled. | Clogged dispense tubes on the washer manifold. | Remove and clean the manifold as described in ***Ch. 4***. |
| | Manifold or tubing not adequately primed. | Run W-DAY_RINSE multiple times. |
| | Dispense flow rate too low. Low flow rate 1 or 2 CW is used with 384-well plates. | Define a higher dispense Flow Rate. |
| | Microplate aspiration height adjustment too high or too low. | Change the aspirate height (Z-axis position) in the protocol. |

| FLUID LEAKAGE | | |
|---|---|---|
| ***Problem*** | ***Possible Cause*** | ***What To Do*** |
| Fluid leaking from manifold. | Defective seals. | Replace the manifold's channel-end seals or o-rings as described in ***Ch. 4***. Contact BioTek TAC. |
| | Aspiration tubes only: vacuum too low. | Check waste connector tubes; make sure they are properly connected.<br>If you are using an in-line vacuum filter, check the filter for clogging, and replace if necessary.<br>Check seal of waste bottle covers.<br>Check for air leaks in the waste tubing and bottles.<br>Use a slower Aspiration Travel Rate. |
| Fluid leaking from underneath the instrument. | Defective tubing connector or inlet tubing. | Contact BioTek TAC. |
| | Leaking valve. | Contact BioTek TAC. |
| Fluid leaking from external tubing connector. | Defective connector. | Replace connector. |
| | Worn tubing. | Replace tubing or cut back tubing one inch (to remove worn section). |
| | Worn seal (inlet or vacuum fitting). | Replace filter or seal. |

| MICROPLATE CARRIER MOVEMENT | | |
|---|---|---|
| ***Problem*** | ***Possible Cause*** | ***What To Do*** |
| Aspiration tubes not entering wells correctly. | Microplate not properly seated or strips not level. | Reseat microplate in carrier or strips in holder.<br>Make sure the carrier is clean.<br>Try a different microplate or strip holder. If the problem is unresolved, the carrier may have to be realigned. Contact BioTek TAC. |
| | Aspirate tubes position is too wide for a movement. | Change the horizontal, X- or Y-axis aspirate position in the protocol. |
| | Aspirate tubes bent. | Contact BioTek TAC. |

| WASHER MANIFOLD MOVEMENT | | |
|---|---|---|
| ***Problem*** | ***Possible Cause*** | ***What To Do*** |
| Manifold position error. | Manifold movement is blocked. | Check orientation of microplate; A1 should be in the left rear corner of the plate carrier as you face the instrument.<br>Check for and remove any obstructions.<br>Ensure the manifold is installed properly. |
| | Incorrect manifold selected. | Make sure the washer manifold setting matches the installed manifold (96-tube or 192-tube). (Tools>Instrument Utilities>Washer>Manifold Selection.) |

| MICROPLATE SCRATCHES | | |
|---|---|---|
| ***Problem*** | ***Possible Cause*** | ***What To Do*** |
| Scratches on microplate bottom. | Microplate dispense or aspiration height adjustment too low. | Change the Dispense or Aspirate height, the Z-axis position. |
| | Microplate not properly seated or strips not level. | Reseat microplate in carrier or strips in holder.<br>Make sure the carrier is clean.<br>Try a different microplate or strip holder. If the problem is unresolved, the carrier may have to be realigned. Contact BioTek TAC. |

## Peri-pump Performance Issues

This table lists possible solutions to potential performance issues when using the Peri-pump dispenser.

| Problem | What To Do |
|---|---|
| Fluid stream missing wells | Check **Tip Holder**, make sure it is properly seated in the Dispense Arm.<br>Select the correct **Plate Type**. |
| Fluid splashing out of the wells | Select the correct **Plate Type**.<br>Reduce the **Flow Rate**.<br>Lower the **Dispense Height**. |
| Uneven dispensing | Make sure all cassette components are properly seated in their respective positions.<br>Tips are clogged. (See the ***Preventive Maintenance*** chapter.)<br>Recalibrate the cassette.<br>Replace the tubing.<br>(On the Operator's Manual CD, in the PDF folder, find instructions for recalibrating the cassette and replacing the tubing.) |
| Dispenser skipping columns | Check/define the **Plate Map**. |
| Tips clogging | Filter the dispense fluid to 50 microns before dispensing.<br>Replace the tubing. |
| Viscous fluids sticking to tips | Vary the **Flow Rate**: experiment with different flow rates to determine which setting best forces fluid to break from the tip. |
| Cannot communicate with computer | Check the cabling. (See previous section.)<br>Select the correct COM Port.<br>Turn on dispenser; display Main Menu. |
| Foaming in the wells | Reduce the dispense step's **Flow Rate**. |

## Syringe Dispenser

| START-UP | | |
|---|---|---|
| ***Problem*** | ***Possible Cause*** | ***What To Do*** |
| Syringe or manifold position error. | Syringe or manifold is being obstructed. | Remove obstruction. |
| | Motor, sensor, or electrical problem. | Turn instrument off, wait at least 15 seconds, and turn it back on. If the instrument does not pass its self-test, contact BioTek TAC. |
| | Syringe piston not seated all the way to the bottom before fastening set screw. | Reinstall the syringe head. |

| SYRINGE MOVEMENT | | |
|---|---|---|
| ***Problem*** | ***Possible Cause*** | ***What To Do*** |
| Syringe position error. | Syringe movement is blocked. | Ensure the 26-pin high-density cable shipped with the Syringe is connected to the EL406's rear panel. |
| | | Contact BioTek TAC. |
| | Syringe piston not seated all the way to the bottom before fastening set screw. | Reinstall the syringe piston and syringe head. |

| FLUID DELIVERY | | |
|---|---|---|
| ***Problem*** | ***Possible Cause*** | ***What To Do*** |
| Unable to dispense fluid. | Inlet tube not connected at manifold or at bottle. | Check all tubing. |
| | Supply tube inside the supply bottle is kinked or disconnected. | Straighten or connect supply tube. Make sure the end is cut as shown in the Optimize Performance section of ***Chapter 3***. |
| | Clogged dispense tubes on the manifold. | Remove and clean the manifold. |
| | Inlet tube is not connected to the bottom port of the syringe. | Connect the inlet tube to the lower port of the syringe pump. |
| | Outlet tube is not connected to the top port of the syringe. | Connect the outlet tube to the top port of the syringe pump. |

| FLUID DELIVERY | | |
|---|---|---|
| ***Problem*** | ***Possible Cause*** | ***What To Do*** |
| | Check valve flow direction is incorrect. | Compare the flow direction of the check valves in ***Chapter 2***. Switch or replace if necessary. |
| | Check valves are stuck closed. | Clean check valves as described in ***Chapter 4***. |
| | No fluid. | Fill bottles with appropriate fluid. |
| | System not primed. | Run S-DAY_RINSE for once or twice for one or both syringes. |
| | Faulty syringe pump. | Contact BioTek TAC. |
| | Set screw not tightened on the syringe pump piston. | Reinstall the syringe head. |
| Plate overfills (floods). | Dispense height too high. | Change the Dispense Z-axis position (height). |
| | Volume too large for the vessel. | Define a smaller volume. |
| | Dispense rate too fast for volume selected. | Define a slower dispense rate or lower volume. |
| Uneven dispensing of fluid; wells not filled. | Clogged dispense tubes on the dispenser manifold. | Remove and clean the manifold as described in ***Chapter 4***. |
| | Manifold or tubing not adequately primed (air in fluid lines). | Run a Prime using 20 mL. Follow with a Dispense: 20 µL per well for 24 strips. |
| | Dispense flow rate too low. | Define a higher flow rate. |
| | Setscrew not tightened on the syringe pump piston. | Reinstall the syringe head as described in ***Chapter 4*** after autoclaving the syringe. |
| | 32-tube dispense manifolds are not dispensing accurately. | "Recalibrate the Backlash" as described in ***Chapter 4*** to determine the optimal backlash setting for the manifolds. |
| Dripping dispense tubes. | Dispense tubing routed incorrectly. | The supply bottle tube must connect to the Syringe's bottom port. |
| Fluid jet is off-center or skewed from 32-tube SB manifold. | Minute particles of debris on the end of the tubes. | Brush away any particles from the end of the tube using a piece of silicon tubing. Silicon will not flake off and leave particles behind like other materials. Alternatively, AutoClean the dispense manifolds as described in ***Ch 4***. |

| FLUID LEAKAGE | | |
|---|---|---|
| ***Problem*** | ***Possible Cause*** | ***What To Do*** |
| Fluid leaking from manifold. | Defective seals. | Replace plugs as described in Chapter 4. |
| | Check valves are leaking. | Clean or replace check valves as described in ***Chapter 4***. |
| | Fittings to manifold are leaking. | Reconnect/reseat the fittings. |
| Fluid leaking from underneath the unit. | Defective syringe cup. | Contact BioTek TAC. |
| | Leaking syringe seal. | |
| | Defective syringe piston. | |
| Fluid leaking from external tubing connector. | Worn tubing. | Replace tubing. |
| | Defective connector. | Contact BioTek TAC. |

| MICROPLATE CARRIER MOVEMENT | | |
|---|---|---|
| ***Problem*** | ***Possible Cause*** | ***What To Do*** |
| Dispense tubes not entering well correctly. | Microplate not properly seated or strips not level. | Reseat microplate carrier, or the plate or strips in holder. |
| | | Make sure the carrier is clean. |
| | Horizontal dispense position does not align the tubes in the wells. | Change the X-axis (horizontal) Position in the protocol. |
| | Dispense tube(s) bent. | Push the supplied stylus into the tube and then gently attempt to straighten the tube using your fingers. If it remains bent, contact BioTek TAC. |
| | Manifold tilted. | Check tubing for twists. |
| Carrier position error. | Carrier movement is blocked. | Check for/remove any obstruction. |
| | Dirty carrier or carrier rail. | Clean carrier and/or carrier rail. |

| DISPENSE MANIFOLD MOVEMENT | | |
|---|---|---|
| ***Problem*** | ***Possible Cause*** | ***What To Do*** |
| Manifold position error. | Manifold movement is blocked. | Check the dispense height or Z-axis positioning. Allow at least 1 mm clearance above plate. |
| | | Check for/remove any obstructions. |
| | | Contact BioTek TAC. |

Appendix A

# Error Codes

A listing of potential error codes and possible solutions for resolving them.

# System Error Codes

Most of these error conditions require technical expertise to correct. Error code 306 and few other exceptions to this rule are listed with remedies in the Troubleshooting section. A few other errors may be caused by an obvious obstruction to a device's movement or insufficient fluid in a supply vessel. Fix these kinds of errors and restart your instrument to give it an opportunity to clear the error code.

Contact BioTek Technical Assistance Center (TAC) for assistance.

| Code | Message | What to do |
|---|---|---|
| 0100 | Task was aborted | Restart instrument if this message is unexpected. |
| 0210, 0220 | Carrier X motor didn't find home opto sensor transition<br>Carrier X motor didn't find autocal jig opto sensor transition | Clean the plate carrier, rails, and glide strips, using mild detergent and hot water, 70% isopropyl alcohol or ethanol. Restart the instrument. If the error occurs again, contact BioTek TAC. |
| 0211, 0221 | Carrier Y motor didn't find home opto sensor transition<br>Carrier Y motor didn't find autocal jig opto sensor transition | Run self test. If error reoccurs, contact BioTek TAC. |
| 0212, 0222 | Dispense head motor didn't find home opto sensor transition,<br>Dispense head motor didn't find autocal jig opto sensor transition | Run self test. If error reoccurs, contact BioTek TAC. |
| 0213, 0223 | Wash head motor didn't find home opto sensor transition,<br>Wash head motor didn't find autocal jig opto sensor transition | Run self test. If error reoccurs, contact BioTek TAC. |
| 0214 | Syringe A motor didn't find home opto sensor transition | Run self test. If error reoccurs, contact BioTek TAC. |
| 0215 | Syringe B motor didn't find home opto sensor transition | Run self test. If error reoccurs, contact BioTek TAC. |
| 0216 | Peri-pump motor didn't find home opto sensor transition | Run self test. If error reoccurs, contact BioTek TAC. |

| Code | Message | What to do |
|---|---|---|
| 0220 | Carrier X motor didn't find autocal jig optical sensor transition. | Service Only. Contact BioTek TAC. |
| 0221 | Carrier Y motor didn't find autocal jig optical sensor transition. | Service Only. Contact BioTek TAC. |
| 0223 | Wash head motor didn't find autocal jig optical sensor transition. | Service Only. Contact BioTek TAC. |
| 0300 | Carrier X motor interlock safety switch open | Service Only. Contact BioTek TAC. |
| 0301 | Carrier Y motor interlock safety switch open | Service Only. Contact BioTek TAC. |
| 0302 | Dispense head motor interlock safety switch open | Service Only. Contact BioTek TAC. |
| 0303 | Wash head motor interlock safety switch open | Service Only. Contact BioTek TAC. |
| 0304 | Syringe A motor interlock safety switch open | Service Only. Contact BioTek TAC. |
| 0305 | Syringe B motor interlock safety switch open | Service Only. Contact BioTek TAC. |
| 0306 | Peri-pump Pump Cover is open | Close the pump cover door and rerun the protocol. |
| 0400 | Carrier X motor failed positional verify | Run self test. If error reoccurs, contact BioTek TAC. |
| 0401 | Carrier Y motor failed positional verify | Run self test. If error reoccurs, contact BioTek TAC. |
| 0402 | Dispense head motor failed positional verify | Run self test. If error reoccurs, contact BioTek TAC. |
| 0403 | Wash head motor failed positional verify | Service Only. Contact BioTek TAC. |
| 0404 | Syringe A motor failed positional verify | Service Only. Contact BioTek TAC. |
| 0405 | Syringe B motor failed positional verify | Service Only. Contact BioTek TAC. |

| Code | Message | What to do |
|---|---|---|
| 0406 | Peri-pump motor failed positional verify | Service Only.<br>Contact BioTek TAC. |
| 0500 | Carrier X motor not homed successfully | Remove any obstructions and restart. If error persists, contact BioTek. |
| 0501 | Carrier Y motor not homed successfully | Remove any obstructions and restart. If error persists, contact BioTek. |
| 0502 | Dispense head motor not homed successfully | Remove any obstructions and restart. If error persists, contact BioTek. |
| 0503 | Wash head motor not homed successfully | Remove any obstructions and restart. If error persists, contact BioTek. |
| 0504 | Syringe A motor not homed successfully | Remove any obstructions and restart. If error persists, contact BioTek. |
| 0505 | Syringe B motor not homed successfully | Remove any obstructions and restart. If error persists, contact BioTek. |
| 0506 | Peri-Pump motor not homed successfully | Service Only.<br>Contact BioTek TAC. |
| 0600-0606 | Specified motor currently in use | Service Only.<br>Contact BioTek TAC. |
| 0700-070F | Invalid motor number specified | Service Only.<br>Contact BioTek TAC. |
| 0900 | Calibration failed. The measured or calculated autocal value is out of tolerance. | Service Only.<br>Contact BioTek TAC. |
| 0A00 | Invalid plate type selected | Service Only.<br>Contact BioTek TAC. |
| 0C01 | Requested config/autocal data absent | Service Only.<br>Contact BioTek TAC. |
| 0C02 | Calculated checksum didn't match saved checksum | Service Only.<br>Contact BioTek TAC. |

| Code | Message | What to do |
|---|---|---|
| 0C03 | Config parameter out of range | Service Only.<br>Contact BioTek TAC. |
| 1001 | Bootcode powerup checksum test failed | Contact BioTek TAC. |
| 1002 | Unknown error in bootcode | Contact BioTek TAC. |
| 1003 | Bootcode page program error | Contact BioTek TAC. |
| 1004 | Bootcode block size error (not 256) | Contact BioTek TAC. |
| 1005 | Invalid processor signature (not 1280,1281,2560,2561) | Contact BioTek TAC. |
| 1006 | Bootcode memory exceeded | Contact BioTek TAC. |
| 1007 | Invalid slave port | Contact BioTek TAC. |
| 1008 | Invalid response from slave | Contact BioTek TAC. |
| 1009 | Invalid processor detected | Contact BioTek TAC. |
| 1010 | Checksum error downloading basecode | Contact BioTek TAC. |
| 1250 | Internal RAM test error on the UI processor | Contact BioTek TAC. |
| 1251 | Internal RAM test error on the MC processor | Contact BioTek TAC. |
| 1260 | Stack test error on the UI | Contact BioTek TAC. |
| 1261 | Stack test error on the MC | Contact BioTek TAC. |
| 1300 | Invalid syringe selection | Contact BioTek TAC. |
| 1301 | Syringe module not connected | Make sure the Syringe module is correctly connected using the new BioTek-provided serial cable. |
| 1302 | Syringe initialization error | |
| 1303 | Syringe sensor not cleared error | |
| 1304 | Invalid syringe dispense volume | See error 1308 below. |
| 1305 | Invalid syringe operation | Contact BioTek TAC. |

| Code | Message | What to do |
|---|---|---|
| 1306 | FMEA error on syringe A | Contact BioTek TAC. |
| 1307 | FMEA error on syringe B | Contact BioTek TAC. |
| 1308 | Invalid Syringe pre-dispense volume | Protocol may have been written for a different type of dispense manifold. Make sure the Instrument Settings represent the installed hardware. Modify the protocol to match. |
| 1309 | Invalid Syringe prime volume | |
| 1310 | Invalid Syringe manifold | |
| 1350 | Peri-pump (PP) invalid dispense volume | Contact BioTek TAC. |
| 1351 | PP invalid cassette type | Change the cassette type to match the protocol requirement and rerun the protocol. |
| 1352 | PP invalid pre-dispense volume | Contact BioTek TAC. |
| 1353 | Multiple required cassettes | Make sure every Peri-pump step in the protocol calls for the same cassette type. A conflict was found. |
| 1354 | PP pump cover (safety door) open | Close the pump cover door and rerun the protocol. |
| 1400 | No vacuum pressure detected after turning on the vacuum pump | Make sure the vacuum pump is turned on, and not leaking; the waste bottle has not overflowed and its caps are seated correctly. If the pump is not running, the fuse may be blown. But, before replacing the fuse, first try to determine and remedy the cause of the failure. If the pump sounds strained or runs slowly, it may be damaged. Review other related Troubleshooting suggestions. |
| 1401 | The waste bottles should be emptied before continuing | Empty the waste bottles and rerun the protocol. |
| 1402 | The requested valve is invalid | Contact BioTek TAC. |
| | | |

| Code | Message | What to do |
|---|---|---|
| 1500 | No buffer fluid detected at the start of a wash protocol | Fluid detection errors. Make sure the supply bottle is full and properly connected, the tubing is not kinked, blocked, etc., and the correct port/valve is selected. |
| 1501 | No buffer fluid detected before the washer dispense step | |
| Note: | Running very low density fluids, like alcohol, may generate some of these fluid detection errors because the fluid prohibits the float detector from operating properly. Disable the Fluid sensor (Setup Menu) while using such low density fluids, just be vigilant about fluid levels. | |
| 1502 | The buffer valve selection is invalid | Contact BioTek TAC. |
| 1503 | Dispense volume error | Contact BioTek TAC. |
| 1504 | No buffer detected flowing through the manifold during an operation | Flow detection errors. Make sure the supply bottle is full and properly connected, the tubing is not kinked, blocked, etc., and the correct port/valve is selected. |
| 1505 | No buffer detected at the end of a wash protocol | |
| 1506 | The requested carrier Y-axis position is out of range | Contact BioTek TAC. |
| 1507 | Internal valve transition error | Contact BioTek TAC. |
| 1508 | Pre-dispense volume error | The specified Pre-dispense volume is invalid for this plate type. Edit the protocol. |
| 1509 | Low flow 192-tube manifold error | The 192-tube manifold does not support cell wash or low-flow protocols. A wash step mismatch has occurred. Edit the protocol. |
| 1510 | Low flow 96-tube manifold error | Contact BioTek TAC. |
| 1511 | External Buffer Switching valve module is required | The current protocol is defined to use different valves of the Buffer Switching module, which is not installed. Edit the protocol. |
| 1513 | Plate type – manifold conflict | Change the Plate Type to one supported by the manifold. Or, click the Instrument Settings link and make sure they match the physical hardware: Get settings from instrument. |

| Code | Message | What to do |
|---|---|---|
| 1600-160C | The onboard storage space allocated for this function has been used up. | Use the LHC “Manage Memory” control to reallocate space. |
| 2400 | Parameter limit exceeded | Contact BioTek TAC. |
| 4000 | Program locked so operation denied | Contact BioTek TAC. |
| 4010 | Program non-erasable so delete denied | Contact BioTek TAC. |
| 4020 | Bad checksum when reading program from EEPROM | Contact BioTek TAC. |
| 4030 | Program not found | Contact BioTek TAC. |
| 4040 | Can’t save program because no space available | Contact BioTek TAC. |
| 4050 | Program run cancelled by user | Restart instrument if this message is unexpected. |
| 8100 | Communications NAK | Contact BioTek TAC. |
| 8101 | Timeout while waiting for serial message data | Contact BioTek TAC. |
| 8102 | Instrument busy and unable to process message | Contact BioTek TAC. |
| 8103 | Receive buffer overflow error | Contact BioTek TAC. |
| 8104 | Checksum error | Contact BioTek TAC. |
| 8105 | Invalid structure type in byMsgStructure header field | Contact BioTek TAC. |
| 8106 | Invalid destination in byMsgDestination header field | Contact BioTek TAC. |
| 8107 | Request object received not supported by instrument | Contact BioTek TAC. |
| 8108 | Message Body size exceeds max limit | Contact BioTek TAC. |

| Code | Message | What to do |
|---|---|---|
| 8109 | Max number of requests currently running and cannot run the latest request | Contact BioTek TAC. |
| 810A | No request running when response request issued | Contact BioTek TAC. |
| 810C | Response for outstanding request not ready yet | Contact BioTek TAC. |
| 810D | To communicate with the LHC, the instrument must be at its main menu | The LHC can only talk to the instrument when its main menu is displayed. When the instrument is busy, for example when AutoPrime is running, press the Stop button on the keypad, if desired, to end the current process and return to the main menu. |
| 810E | One or more request parameters are not valid | Contact BioTek TAC. |
| 810F | The command was received while the software was not ready to accept that command | Contact BioTek TAC. |
| A000 | Task control block not available | Service Only.<br>Contact BioTek TAC. |
| A100<br>-A10F | Software device not available | Service Only.<br>Contact BioTek TAC. |
| A200 | Version strings for multiple microprocessors do not match | Service Only.<br>Contact BioTek TAC. |
| A301 | +5v logic power supply level error | Service Only.<br>Contact BioTek TAC. |
| A302 | +24v system/motor power supply level error | Service Only.<br>Contact BioTek TAC. |
| A303 | +42v Peri-pump motor power supply level error | Service Only.<br>Contact BioTek TAC. |
| A400 | Malloc failed | Service Only.<br>Contact BioTek TAC. |

| Code | Message | What to do |
|---|---|---|
| A500 | Multiple tasks attempted to use display simultaneously | Service Only.<br>Contact BioTek TAC. |
| A600 | Serial EEPROM access error | Service Only.<br>Contact BioTek TAC. |

# EL406 IS Error Codes

The EL406 Interface Software (IS) can generate the following error codes when you are using the LHC to control the instrument. Generally, these errors are caused by protocol parameters that conflict with the instrument's onboard settings. The protocol may have been originally created for a different hardware configuration, the 192-tube wash manifold instead of the 96-tube, for example.

**Quick Fix:** Make sure your **Instrument Settings** accurately reflect your instrument's hardware configuration and then, modify the protocol to fix any invalid parameters. With the EL406 connected to and communicating with your computer and its main menu displayed on the keypad:

1. Click the Settings link in the main view.
2. In the **Instrument Settings** dialog, click the instrument link to get the settings from the instrument.
3. Modify the protocol step that generated the error message.

| Error Code | Description | Help |
|---|---|---|
| 6000 | General communication error during download. | See *Communication Errors: EL406 to Computer* in the LHC Help. |
| 6001 | COM port created by USB converter no longer active | See the LHC Help topic: *About COM Ports.* |
| 6002 | Invalid basecode part number; instrument is not an EL406 | Service Only.<br>Contact BioTek TAC. |
| 6003 | Invalid Basecode Data Version; basecode needs to be updated | Contact BioTek to obtain latest basecode. |
| 6010 | The data is invalid or out-of-range. | Service Only.<br>Contact BioTek TAC. |
| 6011 | This step type cannot be downloaded. | Review the limitations to transferring protocols to the instrument, See the LHC Help Topic: *Transferring Protocols*. |
| 6012 | Illegal characters in protocol name | See the LHC Help topic: *Define a Protocol.* |
| 6013 | The protocol name length must be 16 characters or less. | |

| Error Code | Description | Help |
|---|---|---|
| 6014 | A 1536 well plate is not supported by this instrument. | Service Only.<br>Contact BioTek TAC. |
| 6015 | The specified volume exceeds the cassette maximum limit. | Modify the volume or change the cassette type. |
| 6016 | The volume is out-of-range. | Modify the volume or change the cassette type. |
| 6017 | Invalid flow rate. | Learn about the *Syringe Dispense Step* in the LHC Help. |
| 6018 | Invalid number of pre-dispenses. | Service Only.<br>Contact BioTek TAC.<br>These codes indicate an unexpected software error that cannot be fixed without BioTek support. |
| 6019 | Invalid horizontal dispense position. | |
| 6020 | Invalid dispense height. | |
| 6021 | Invalid plate clear height. | |
| 6022 | Invalid column selection value (must be 0 or 1). | |
| 6023 | Invalid protocol step type. | |
| 6024 | The Definition String contains invalid data. | |
| 6025 | Manifold conflict between protocol requirements and instrument configuration. | Change the Washer Manifold or change the Instrument Setting. See ***Appendix B***. |
| 6026 | Valve module conflict between protocol requirements and instrument configuration. | Make sure the Buffer Switching setting matches your instrument; see Instrument Settings. |
| 6027 | Syringe module conflict between protocol requirements and instrument configuration. | Make sure the Syringe dispenser setting matches your instrument: see Instrument Settings. |
| 6028 | Filter washer conflict between protocol requirements and instrument configuration. | Service Only.<br>Contact BioTek TAC. |
| 6029 | Required cassette does not match installed cassette. | Change the cassette type to match the protocol requirement and rerun the protocol. |

| Error Code | Description | Help |
|---|---|---|
| 6030 | Invalid cassette type was specified. | Service Only.<br>Contact BioTek TAC. |
| 6031 | Cannot use a 96-well plate with a 192-tube manifold. | Modify the Plate Type or Change the Washer Manifold. |
| 6032 | Downloading Protocols is not supported. | Service Only.<br>Contact BioTek TAC. |
| 6033 | This step is not supported for 1536-well plates. | Fix the plate type or the Instrument Settings. A conflict between the plate type and installed hardware devices has been detected.<br>Change the Plate Type to one supported by the washer/dispenser. Or, click the Instrument Settings link and make sure they match the physical hardware: Get settings from instrument. |
| 6034 | The 32-tube Syringe Manifold is required for 1536-well plates. | |
| 6035 | The 16-tube Syringe Manifold is required for 96- and 384-well plates. | |
| 6036 | The 128-tube Washer Manifold is required for 1536-well plates. | |
| 6037 | The 128-tube Washer Manifold can only be used for 1536-well plates. | |
| 6038 | This step only applies to 1536-well plates. | |
| 6039 | Conflicting column selection | Fix the plate map (selected columns to dispense to). A protocol parameter may have been changed after a partial plate dispense was defined. |
| 6040 | Invalid baud rate | Service Only.<br>Contact BioTek TAC.<br>These codes indicate an unexpected software error that cannot be fixed without BioTek support. |
| 6041 | Invalid data bits selection | |
| 6042 | Invalid stop bits selection | |
| 6043 | Invalid parity selection | |
| 6044 | Serial port error | Fix the COM port setting. Check the cabling. Click the Port link and use the drop-down menu to see all active ports. Customize the Pre-defined Protocols to avoid this error in future. |
| 6045 | Serial write error | |
| 6046 | Serial read error | |

| Error Code | Description | Help |
|---|---|---|
| 6047 | Checksum error | Contact BioTek TAC. |
| 6048 | Serial NAK error | Make sure the COM port setting is correct and the cable is properly connected. Restart the instrument. If error reoccurs, contact BioTek TAC. |
| 6049 | Excess data, or not enough data, received. | Reset the instrument. Try running a different protocol. If error reoccurs, contact BioTek TAC. |
| 6050 | Invalid message header | |
| 6051 | Invalid message object | |
| 6052 | Invalid message body size | |
| 6053 | Serial message timeout | |
| 6054 | Port handle error | |
| 6055 | Read timeout value is invalid. | |
| 6056 | Unauthorized to open the COM port | Make sure the COM port setting is correct and the cable is properly connected. Restart the instrument. If error reoccurs, contact BioTek TAC. |
| 6057 | Out-of-range parameter for the open port function. | |
| 6058 | Unable to open the COM port. | |
| 6059 | Unable to clear the transmission buffer. | |
| 6060 | Unable to close the port. | |
| 6061 | Port is no longer available. | |
| 6070 | Invalid Syringe specified. | Service Only.<br>Contact BioTek TAC.<br><br>These codes indicate an unexpected software error that cannot be fixed without BioTek support. |
| 6071 | Invalid number of syringe prime cycles | |
| 6072 | Invalid syringe Aspirate Delay value | |
| 6073 | Invalid X-axis offset value | |

| Error Code | Description | Help |
|---|---|---|
| 6074 | Invalid Y-axis offset value | Service Only.<br>Contact BioTek TAC.<br><br>These codes indicate an unexpected software error that cannot be fixed without BioTek support. |
| 6075 | Invalid Z-axis offset value | |
| 6080 | Invalid Peri-pump prime duration | |
| 6085 | Invalid minutes:seconds value | |
| 6086 | Invalid hours:minutes value | |
| 6088 | Invalid Shake/Soak options selected | |
| 6089 | Invalid Shake Intensity selected | |
| 6090 | Invalid Washer buffer selected | |
| 6091 | Invalid Washer Aspirate Delay value | |
| 6092 | Invalid Washer Aspirate Travel Rate value | |
| 6093 | Invalid Wash Cycles value | |
| 6094 | Invalid Wash format selected | |
| 6095 | Invalid Wash Sectors selected | |
| 6096 | Wash Aspirate Delay value is required. | |
| 6097 | Syringe Dispense Volume must be an integer. | |
| 6098 | Peri-pump cannot run with the pump cover open. | Close the pump cover door and rerun the protocol. |
| 6100 | This functionality requires the software to be registered. | You must register both the LHC and the EL406 IS software with BioTek. See LHC installation guide. |

Appendix B

# Changing the Washer Manifold

Instructions for changing the washer manifold: accessory manifolds, 192-tube for processing 384-well plates and 128-tube aspirate manifold for processing 1536-well plates, replace the standard 96-tube manifold.

# Changing the Manifold

Three wash manifolds are supported by the EL406: 96-, 192-, and 128-tubes. All three manifolds fit onto the instrument and are removed from it in a similar manner. Accessory manifolds are shipped in special packaging. Use the shipping case to store whichever manifold is not being used.

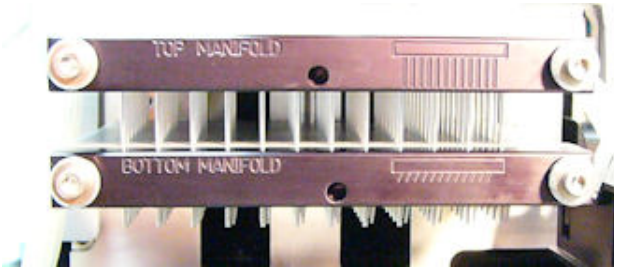
Before removing the installed manifold, run a Maintenance protocol (such as W-DAY_RINSE) or the "Long_Shutdown" procedure described in the ***Maintenance*** chapter to flush any remaining residue from the manifold tubes, if the washer has been in operation.

> ❖ **Tip:** It is easier to clean the manifold before removing and storing it than afterwards when residuals in the tubing and fluid paths have been allowed to dry or crystallize during storage.

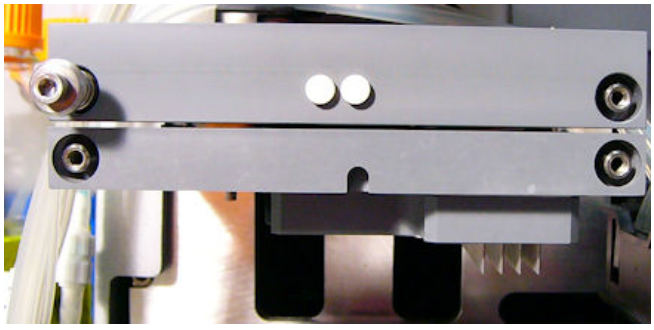
### First, clean the manifold:

1. Run W-DAY_RINSE one or two times with deionized water in the supply bottle.
2. Run the system "dry":
    1. Disconnect the fluid supply from the instrument,
    2. Run W-DAY_RINSE.
3. Turn off the instrument and disconnect the power cable.

> ❖ Alternatively, use the **Ultrasonic Advantage™:** Run AutoClean instead of W-DAY_RINSE.




Dual 96-tube manifold



128-tube manifold

### Replace the manifold:

1. Remove the mist shield.

2. Using the 9/64" (3.57 mm) hex wrench supplied with the instrument, remove the screws, washers, and springs that hold the manifold in place, and set them aside.
3. Carefully remove the manifold and end plates, holding the upper and lower manifolds together as a single unit; the 128-tube manifold is a single unit and does not have end plates. Place the manifold into the shipping case for safe storage (see photos on next page). If you ran W-DAY_RINSE or an AutoClean protocol as instructed, ensure that the manifold is thoroughly dry before storing it.
4. Install the alternate manifold and end plates, if applicable, carefully holding the upper and lower manifolds together as a single unit, and making sure that the two o-rings do not fall out of their grooves during installation. **Do not over-tighten the manifold mounting screws.**

**Important!** When reinstalling the manifold, only tighten the screw-washer-spring assembly that holds it in place until you feel the mechanical stop. Tightening past this point will damage the instrument and will **void your warranty.**

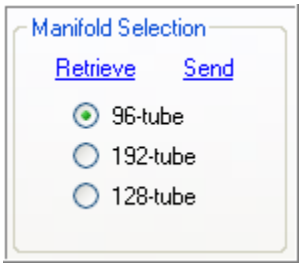
### Reinstall the mist shield:

1. Align the mist shield with the washer so it rests on top of the two posts and the two thumbscrew holes in the shield are lined up with the two holes in the base of the washer.
2. Insert the two thumbscrews and **finger-tighten only**.

### Reconfigure the instrument:

Configure the washer for operation with the correct manifold:

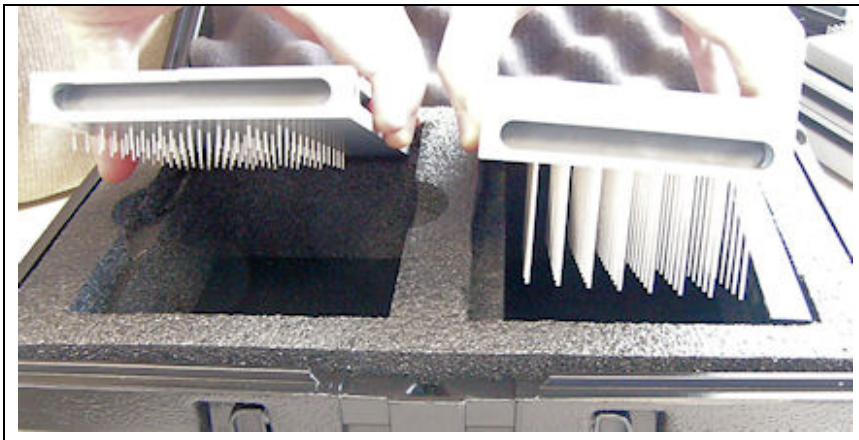
1. Using the LHC, select **Tools> Instrument Utilities> Washer**




2. Under Manifold Selection, choose the button that represents the installed manifold.
3. Click **Send** to send this setting to the EL406™.

**Important!** The correct manifold must be chosen as the **Manifold Selection** before operating the washer. Failure to set the manifold type before operation may damage the manifold and void your warranty.

## Storing the Unused Manifolds



96- and 192-Tube Boxes

Separate the dual manifolds to store them. Put the bottom manifold in the left recess and the top manifold in the right recess.

128-Tube Box

Hold the dual manifolds together and gently lower them into the single recess of the 128-tube manifold box. Then place the end plates, one at a time, into the same recess, on the left side of the manifolds. All components will fit snugly into place.

Appendix C

# Chemical Compatibility

A listing of the materials used in the EL406™ and how they react to commonly used chemicals.

# Chemical Compatibility

For your own safety and to ensure the EL406's long life, follow these guidelines when choosing compounds to use.

> ❖ **Important:** We advise against continuous contact with harsh chemicals. Best practice is to rinse all components with deionized water after contact with any strong acid, base, or solvent.

An Excel® spreadsheet containing this information is provided on the EL406™ Operator's Manual CD in the Spreadsheets folder. You may find it easier to use.

The following materials are used in the EL406. Materials listed as 1-20 in **Table 1** are cross-referenced as headings 1-20 in **Table 2**. Table 1 indicates which component contains the material using **W** for washer, **P** for Peri-pump, and **S** for the dual Syringe dispensers.

### Table 1. Material / Where Used List

| # | Material | | Where Used |
|---|---|---|---|
| 1 | 304 Stainless Steel | W | Inlet screen, feeder tube to manifold, vacuum switch, fluid pump |
| 2 | 316 Stainless Steel | W | Fluid inlet filter, feeder tube to manifold, vacuum switch, fluid pump |
| | | P | Steel dispense tips, pump rollers, carrier rails, cassette bolts |
| | | S | Dispense tubes, syringe piston, cylinder head and fittings, check valve spring |
| 3 | Aluminum | | Instrument Base pan, main structural plate |
| | | P | Center Holder, Tube Tensioner, pump head, dispense arm |
| 4 | Borosilicate Glass | S | Syringe cylinder, supply bottles |
| 5 | Brass | P | Threaded inserts in Center Holder and Tip Holder |
| 6 | EP (Ethylene Propylene) | W | Inlet valve, vacuum switch |
| | | S | Check valve o-rings (organic solvent tubing set) |
| 7 | ETFE (Ethylene tetrafluoroethylene) | S | Manifold fitting |
| 8 | Neoprene | W | Manifold channel-end seals |
| 9 | Nylon | W | Inlet fitting, vacuum switch adjustment screw, carrier leveling feet |
| 10 | Polycarbonate | | Plate carrier |
| | | W | Vacuum switch |
| | | P | Microplate clamp, cassette rest |
| 11 | Polyethylene | W | Buffer Bottle |
| 12 | Polypropylene | W | Outlet fitting, fittings in bottles, inline fittings, float ball |
| | | P | Molded dispense tips |

| # | **Material** | | **Where Used** |
|---|---|---|---|
| | | S | Bottle cap and fittings, check valves (organic solvent tubing set) |
| 13 | Polystyrene | | Assay plates, Mist shield |
| | | W | Flow sensor |
| | | P | Peristaltic pump cover, RFID antenna cover |
| 14 | PTFE (polytetrafluoro-ethylene) | W | Optional check valves (PN: 68098) for fluid pump |
| | | S | Syringe seal |
| 15 | CPVC (Polyvinyl chloride) | W | Inlet screen, inlet valve, manifold, waste sensor, flow sensor |
| 16 | Ryton® PPS | W | Outlet valve, fluid pump, CW inlet valve |
| | | P | Tube Organizer, priming trough, priming trough insert |
| | | S | Manifold, manifold plugs, priming trough insert |
| 17 | Santoprene | W | Fluid pump |
| 18 | Silicone | W | Inlet tubing, outlet tubing, o-rings |
| | | P | Tubing |
| | | S | Tubing, manifold o-rings, check valve o-rings |
| 19 | Ultem (polyetherimide) | W | Outlet valve, inlet valve, buffer switching valves |
| | | P | Center Holder, Tube Tensioner, Tip Holder |
| | | S | Check valves |
| 20 | Viton | W | Outlet valve |

## Table 2. Chemical Compatibility Ratings

| Key | |
|---|---|
| A | No effect |
| B | Slight effect |
| C | Moderate effect |
| D | Severe effect |
| ND | No data |

Table 1. Materials abbreviated

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 304 SS | 316 SS | Alumin. | BoroGlas | Brass | EP | ETFE | Neopren | Nylon | Polycarb | Polyethy | Polyprop | Polystyr. | PTFE | CPVC | Ryton | Santopr. | Silicone | Ultem | Viton |

| Chemical | (1-20 represent Materials listed in ***Table 1*** on the previous pages) | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| Acetic Acid, 5% | B | A | B | A | D | B | C | ND | A | D | A | A | D | A | D | A | A | A | A | B |
| Acetic Anhydride | B | A | A | A | D | A | A | A | A | B | D | B | D | A | D | A | A | A | ND | D |
| Acetonitrile | ND | A | B | A | ND | C | A | D | D | C | D | A | D | A | D | A | ND | D[1] | D | D |
| Ammonia 10% | A | A | A | A | ND | A | A | B | A | A | D | A | B | A | B | A | A | D | D | D |
| Benzyl Alcohol | B | B | B | A | ND | D | A | B | B | D | ND | A | D | A | D | A | A | A | ND | A |
| Chloroform | A | A | B | A | B | A | A | C | D | B | D | C | D | A | D | A | D | D | D | A |
| Detergents 1% | A | A | B | ND | ND | C | D | B | C | D | A | A | A | A | A | A | B | A | A | A |
| Dimethylformamide | A | B | A | A | ND | A | A | B | A | A | D | A | D | A | D | A | D | A | ND | C |
| DMSO (Dimethylsulfoxide) | ND | A | A | A | ND | A | A | B | D | A | D | A | D | A | D | A | ND | C[1] | D | D |
| Ethyl Alcohol 70% | A | A | A | A | A | A | A | A | B | B | B | A | A | A | B | A | B | B | A | B |
| Ethylene Oxide | B | B | D | A | D | ND | A | D | C | D | C | D | C | A | C | D | ND | A | ND | C |
| Formaldehyde 37% | A | A | B | ND | A | A | A | B | B | A | A | A | ND | A | A | A | ND | C | A | A |
| Hexane | A | A | A | ND | A | ND | ND | C | A | B | D | B | D | A | B | A | ND | D | A | A |
| Hydrocholoric Acid 20% | D | D | D | ND | D | ND | A | A | D | A | B | B | C | A | A | D | A | D | A | A |
| Hydrofluoric Acid 20% | D | D | D | A | ND | B | A | B | A | A | D | A | ND | A | B | A | A | D | ND | A |
| Hydrogen Peroxide 10% | B | B | A | A | D | B | A | C | A | C | A | A | A | A | A | C | ND | A | A | A |
| Isopropyl Alcohol 70% | B | A | A | A | A | B | A | C | A | C | A | A | A | A | B | A | ND | A | A | A |
| Methyl Alcohol 70% | A | A | A | A | A | A | C | B | A | A | B | A | ND | A | A | A | B | A | A | A |
| Methylene Chloride | B | B | C | ND | A | ND | A | D | C | ND | D | B | D | A | D | A | D | D | D | B |
| Phosphoric Acid 10% | D | D | C | A | D | A | ND | ND | A | A | A | A | B | A | B | A | A | C | A | A |
| Propylene Glycol | B | B | B | A | ND | B | C | ND | A | D | B | A | A | A | C | A | ND | A | ND | A |
| Sodium Chlorate | A | B | C | A | ND | A | A | A | A | B | A | A | ND | A | A | A | A | C | ND | A |
| Sodium Hydroxide 20% | B | B | D | A | B | C | A | D | D | C | A | A | A | A | A | A | ND | A | A | A |
| Sodium Hypochlorite <20% | C | C | D | A | D | A | A | B | A | A | C | A | A | A | A | C | ND | B | B | A |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sodium Hypochlorite 0.5% | B | B | D | A | D | D | A | B | B | D | C | A | A | A | A | C | ND | B | A | A |
| Sulfuric Acid <10% | D | B | B | A | ND | A | A | C | D | B | A | A | A | A | A | A | A | C | A | A |
| Trichloroethylene | B | B | D | ND | ND | C | D | B | C | D | ND | C | D | A | D | A | D | D | D | A |
| Virkon 10% | ND | A | D | A | ND | A | A | B | A | A | A | A | A | ND | A | A | ND | A | ND | A |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| | 304 SS | 316 SS | Alumin. | BoroGlas | Brass | EP | ETFE | Neopren | Nylon | Polycarb | Polyethy | Polyprop | Polystyr. | PTFE | CPVC | Ryton | Santopr. | Silicone | Ultem | Viton |

- (1) Exposure to DMSO and Acetonitrile may cause the silicone tubing to swell, increasing the volume of fluid dispensed. The magnitude of this effect will vary with concentration and exposure. Re-calibration of the cassette may be required.
- Please contact BioTek with questions about compatibility of any chemicals not described here.