PHERAstar® *FSX*

The gold standard for high-throughput screening

PHERAstar *FSX*

BMG LABTECH

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The Microplate Reader Company

Your benefits at a glance:

- Best sensitivity and speed in all assays
- Assay Optic Modules for easiest optical configuration
- High temporal resolution in TR-FRET detection
- Cross-talk reduction for AlphaScreen and luminescence

- Steady temperature unaffected by external changes
- Automation friendly
- Optimisation tool for TRF/TR-FRET and AlphaScreen
- Made-in-Germany dependability

The best performance in all assays

Developed to fulfil the hardest High-Throughput Screening (HTS) requirements, the PHERAstar® *FSX* high-end micro-plate reader meets the needs of every modern laboratory. Specifically conceived for the highest sensitivity and the fastest speed, its unique features make it superior to any other microplate reader currently on the market.
High-end German engineering combined with the latest technologies makes the PHERAstar *FSX* the gold standard microplate reader for HTS.
This multi-mode plate reader performs all the leading detection technologies:

- UV/vis absorbance
- Fluorescence intensity, including FRET
- Luminescence (flash and glow), including BRET
- Fluorescence polarization/anisotropy
- Time-resolved fluorescence (TRF), including TR-FRET
- AlphaScreen®, AlphaLISA® and AlphaPlex™

Sensitivity and speed

Whatever your application, the PHERAstar *FSX* combines the fast read times necessary for HTS with the sensitivity to read small volumes in all plate formats up to 3456 wells. In single flash mode, the reader can measure a 1536-well plate in 27 seconds - one of the fastest readers on the market. Even at low concentrations and small assay volumes, the unsurpassed sensitivity of its detection system provides outstanding signal-to-noise, %CV, and Z' values.

Innovative technology

The outstanding sensitivity of the PHERAstar *FSX* is centred on an innovative optical design composed of a free air optical path, three independent light sources, Simultaneous Dual Emission detection, and high transmission filters. The optical system is able to adjust the detection focal height for both top and bottom readings to ensure the highest possible sample signal. Top or bottom detection can be directly selected by a mouse click, requiring no hardware changes. Depending on the application, users can choose one of the following light sources for their measurements:

- High energy xenon flash lamp
- Laser for TRF/TR-FRET
- Laser for AlphaScreen, AlphaLISA and AlphaPlex

Four photomultiplier tubes, combined in two application dedicated matched pairs, are used as detectors. One pair is dedicated to simultaneous dual fluorescence and luminescence detection, the second to TRF-based signals.

Switch between assays in seconds

Never worry about which filters or dichroic mirrors are installed. Our application-specific Optic Modules make the PHERAstar *FSX* the easiest reader to optically configure on the market. Optic Modules contain all the components required for a specific assay such as excitation and emission filters, dichroic mirrors, beam splitters and polarization filters. Modules are automatically recognized via barcode and selected by the reader for the appropriate assay. You can easily add or replace them within seconds.

Cut read times in half

Numerous assays such as FRET, TR-FRET, BRET, FP and AlphaPlex™ require detection of two emission wavelengths. Thanks to Simultaneous Dual Emission (SDE) detection, the PHERAstar *FSX* can simultaneously detect two separate emission wavelengths in one single measurement. SDE detection can be used in any assay that measures two emission wavelengths or polarization vectors. It offers a significant speed advantage by cutting read times in half and eliminates the typical drawbacks of double sequential detection such as flash-to-flash variations, photobleaching and decaying kinetic signals. SDE also reduces the variability caused by pH or temperature fluctuations and by evaporation.

Easy assay setup

The Enhanced Dynamic Range (EDR) technology grants a dynamic range spanning over 8 concentration decades in a single measurement, significantly simplifying detection setup. EDR can be applied in top or bottom fluorescence intensity and luminescence detection. Its benefits include:

· Every plate is automatically read with a setting that provides the best sensitivity and signal-to-blank ratios
· Almost any sample is always within dynamic range
· Kinetic assays with signal intensities increasing over time do not saturate the detector
· Assays with very low and very bright signals on the same plate can be measured in the same run
· Data acquired at different times are comparable as count scales are uniform from day to day and plate to plate.

Ultra-fast detection of absorbance spectra

Full-spectrum detection can highlight shifting peaks or the presence of contaminants. Our spectrometer captures a full absorbance spectrum (220 - 1000 nm) at resolutions selectable from 1 to 10 nm in less than 1 second/well. Alternatively, up to 8 discrete wavelengths can be acquired simultaneously. For DNA quantitation, 260-, 280- and 340 nm measurements are all captured with a single flash.


Assay-specific Optic Modules, fully-equipped, installed in seconds.

Without aperture | With aperture
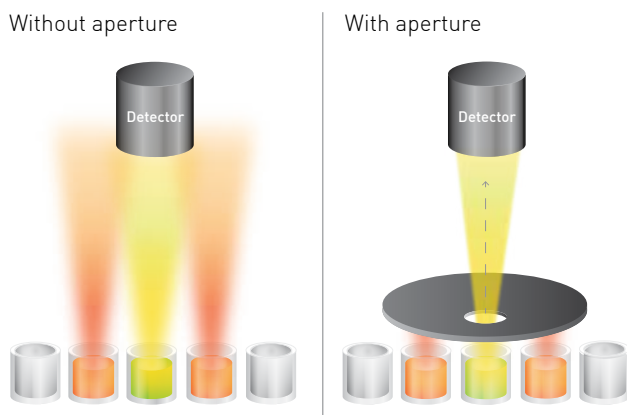
The cross-talk reduction package reduces unspecific light signals that are cause of higher background noise and variability.

Cross-talk reduction

AlphaScreen/AlphaLISA and glow luminescence assays in 384- and 1536-well plates in particular are often negatively affected by stray light and cross-talk. Unwanted light may diffuse from adjacent wells to the detector, as well as through the plastic walls of the wells. If cross-talk is not reduced, low-signal wells might see more counts from nearby bright wells than from their actual signal. BMG LABTECH's cross-talk reduction package auto-matically applies an aperture to physically reduce non-specific signals diffusing from nearby wells, and mathematically corrects for light transmitted through the walls of a well.
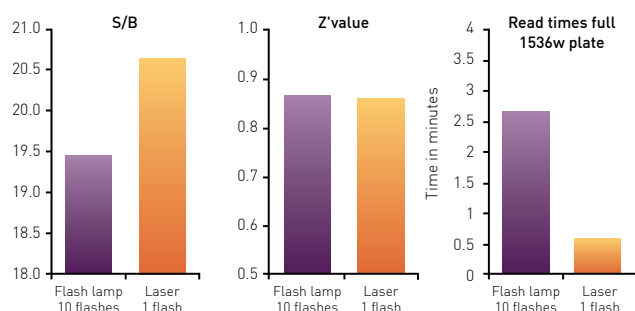

Comparison of xenon lamp (10 flashes) and TRF laser (1 flash) for Signal-to-Blank (S/B), Z' value and read times with a TR-FRET assay.

Dedicated excitation lasers

The PHERAstar *FSX* is equipped with dedicated lasers for the excitation of TRF and Alpha Technology assays. Dedicated lasers significantly improve assay performance and lower limits of detection as they yield a higher excitation energy at a specific wavelength.
The TRF laser specificaly excites samples at 337 nm. With 60 laser flashes per second, it allows for ultra-fast temporal resolution in TR-FRET measurements and even "flying mode" detection. Measurements can take place with a single laser flash without stopping plate movement, significantly reducing read times for an entire plate.
The Alpha Technology laser specifically excites beads at 680 nm, providing a broad dynamic range and an increased signal-to-noise ratio for all AlphaScreen, AlphaLISA and AlphaPlex assays.
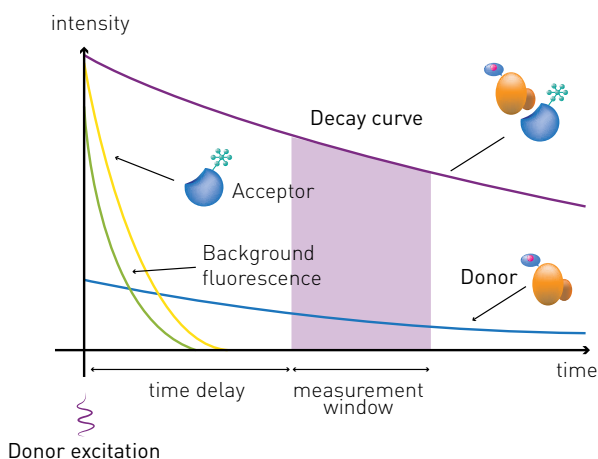
Unique TRF Decay Curve Monitoring feature for assay optimisation.

Fine-tune your signal detection

Decay Curve Monitoring (DCM) is a unique feature of the PHERAstar *FSX* and a fundamental tool for assay development and performance fine-tuning in TRF, TR-FRET and Alpha Technology.
Enabled by a dedicated photon counting detection system, DCM measures and displays the time-resolved emission curve of the fluorophore. The TR-FRET dedicated photon counting detection system enables simultaneous monitoring of both donor and acceptor decay curves with a time resolution of 2 μs. Combined with the Integration Time Wizard, DCM helps to refine timing parameters, thus improving signal detection and reducing background noise.

Advanced Well Scanning

Conventional readers excite and measure samples in the centre of the well. This can negatively affect data quality when measuring uneven cell layers or non-homogenous well samples. On the PHERAstar *FSX*, three different well scan modes enable robust data acquisition even from non-homogeneous samples such as adherent cells or bacteria. Orbital and spiral averaging automatically normalise for heterogeneous cell distributions. In these modes, the reader takes several measurements on a defined orbit and calculates an average for each well.

For higher resolution, matrix scan acquires up to 900 data points/well, displays each scan point graphically and creates a map for each well. Single scan points or entire sections can be easily removed upon detection.
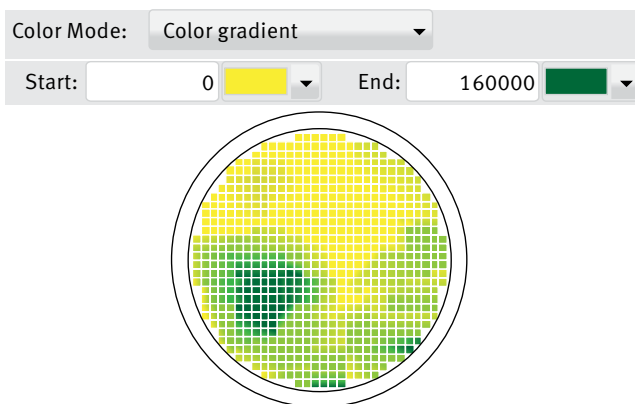
Matrix scanning of GFP-expressing protoplasts. The scan shows how protoplasts (green colour) are not evenly distributed in the well.

Unaffected by environmental changes

The new Advanced Assay Stability (AAS) system enables the PHERAstar *FSX* to match room temperature conditions and to be maintained at any temperature between 18 and 45°C. It achieves this by automatically heating or cooling the measurement chamber depending on the external environment. The AAS system provides a steady temperature that is unaffected by external environmental changes while the plate reader is operating, providing improved assay stability and more reliable data. The special design of the AAS system ensures that all plates in a screening campaign are measured under the same conditions. It provides a consistent environment for temperature-sensitive and kinetic assays, and reduces sample evaporation compared to conventional plate readers.
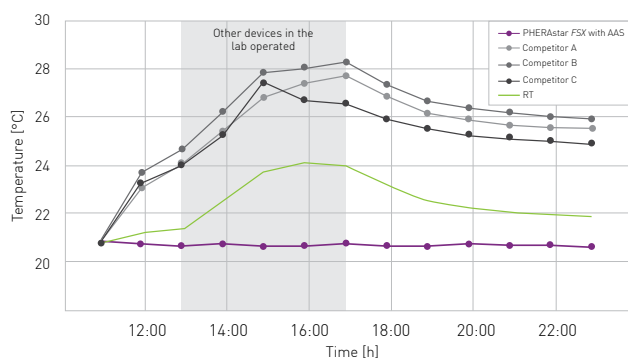
The AAS system provides a stable temperature inside the PHERAstar *FSX* regardless of external temperature fluctuations (gray box).

Smart reagent injection

Reagent injection can be used to add a stimulus or inhibitor, and to initiate or stop kinetic and enzymatic reactions while detecting in real time the sample's reaction. On the PHERAstar *FSX*, two built-in reagent injectors allow for simultaneous detection and reagent injection. This ensures that even fast kinetic reactions are monitored without the loss of any data point. Injectors are readily accessible and housed within the instrument to protect any light-sensitive reagent. Extremely small dead volume and back flushing significantly reduce wastage of expensive reagent. The number of injections/well and injection volumes are adjustable for each well, allowing you to automatically produce dilution schemes and concentration gradients across the microplate.

The PHERAstar *FSX* can be equipped with a Stacker for mid-throughput applications.

MARS data analysis software for automated data reduction.

NanoBRET competitive binding experiments of propranolol-BY630 with increasing concentrations of known unlabelled ß2AR ligands.

Proof of performance of an AlphaLISA-based SARS-CoV-2 Spike Protein - ACE2 competitive binding assay.

Data analysis made easy

The software package includes the Reader Control and MARS data analysis interfaces. This multi-user software can be installed on as many computers as you require, without the need to purchase additional licenses.
The Reader Control software allows to define measurement protocols and acquire data. It is an extremely versatile interface for the straightforward execution of routine tasks, as well as the optimisation of complex operations.
MARS is designed to make data analysis simple and effective, and offers multiple data reduction possibilities such as:

· Standard Curve Wizard for a step-by-step standard curve calculation
· Automatic DNA/RNA concentration determination
· Data display as bar charts, box plots, violin plots etc.
· UV/vis spectral view and analysis
· Background and baseline correction
· Signal interpolation: linear or cubic spline
· Various curve fit models including linear, 4-/5-parameter, polynomial and user-defined fit models
· Enzyme kinetic analysis using various models
· EC_{50} calculation with confidence intervals
· Binding rates and constants determination
· ANOVA, Student's t-test or multiple comparisons
· Performance evaluation: signal-to-blank, signal-to-noise, %CV, Z-prime, etc.
· Automatic data processing using predefined templates

The software package comes with flexible data export (Excel, ASCII) and integration capabilities, and is fully compliant with FDA regulation 21 CFR Part 11.

Applications hub

A perfectly engineered instrument is only part of the solution, it needs to effectively perform all of the leading applications. We continuously work with all major reagent companies to develop protocols and improve instrument settings for their existing assays and their newest kits. The PHERAstar *FSX* is a user-friendly and flexible instrument that provides excellent performance in all applications, including NanoBRET™, AlphaLISA and TR-FRET assays, as shown in the examples. Applications include:
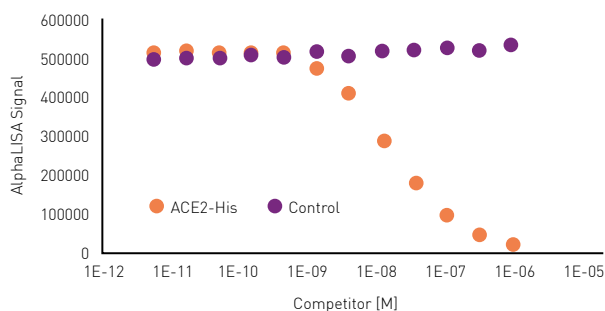
· Protein-protein interactions
· Kinetic binding assays
· Compound and inhibitor screening
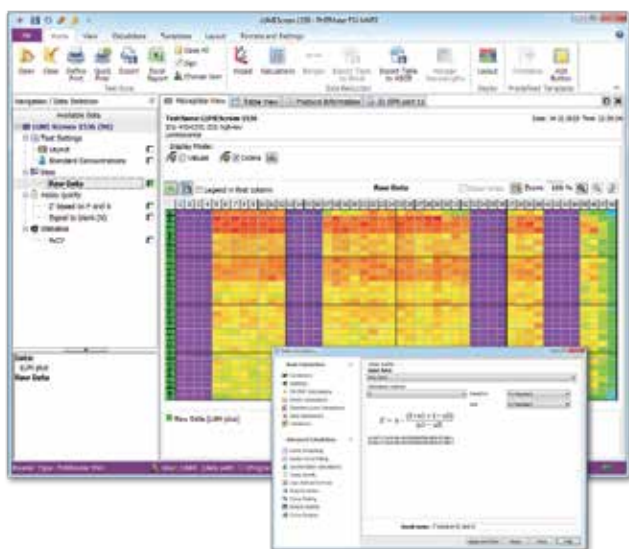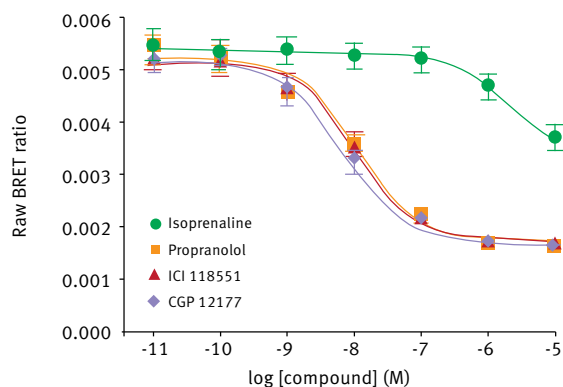
- DNA, RNA, and protein quantification
- Enzyme activity and kinetic assays
- Cell-based assays
- And much more ...

Our comprehensive online application database reflects more than 30 years of expertise and innovations. Over 12,000 published entries of peer-reviewed articles and application notes demonstrate the flexibility and versatility of our readers, and their use in chemical and biological sciences.
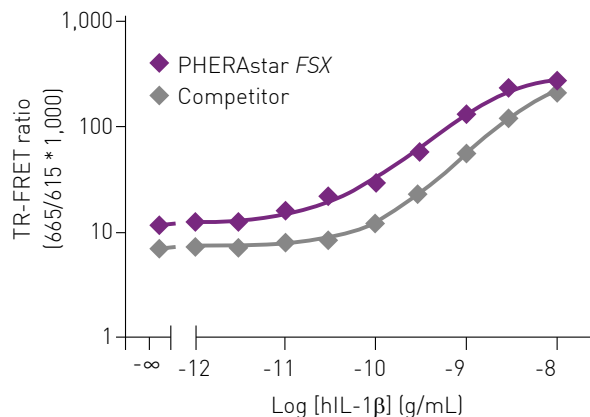

TR-FRET comparison between the PHERAstar *FSX* and another HTS microplate reader, using a THUNDER human IL-1ß standard curve.

Automation

The PHERAstar *FSX* offers excellent robotic integration capabilities, multi-user control, digital signature and FDA 21 CFR part 11 compliance. Its robotic software interface make it easy to integrate into all leading robotic platforms. The instrument comes with three integrated microplate barcode readers and can be equipped with a Stacker.

Support and training

BMG LABTECH operates globally through an extensive network of subsidiaries and trained distributors. Customers can rely on qualified support and assistance with regard to software, assay development, or general enquiries related to the PHERAstar *FSX* and all our other microplate reading solutions.

Easy integration into all leading robotic platforms.

AlphaTechnology includes AlphaScreen, AlphaLISA, and AlphaPlex. These are registered trademarks of Revvity, Inc.
HTRF is a registered trademark of Cisbio Bioassays.
LanthaScreen is a registered trademark of Thermo Fisher Scientific.
Transcreener is a registered trademark of Bellbrook Labs.
DLR and NanoBRET are trademarks of Promega Corp.
Mycoalert is a registered trademark of Lonza.
THUNDER is a trademark of Bioauxilium.

The PHERAstar *FSX* can include all or any combination of features/options/accessories listed below at purchase. Upgrading with additional features/options/accessories may be possible after purchase. Contact your local representative for more details or a quote.

Detection modes	UV/vis absorbance Fluorescence intensity - incl. FRET Luminescence (flash and glow) - incl. BRET Fluorescence polarization Time-resolved fluorescence TR-FRET AlphaScreen®/AlphaLISA®/AlphaPlex™
Measurement modes	Top and bottom reading Endpoint and kinetic Sequential multi-excitation Sequential multi-emission Simultaneous Dual Emission Spectral scanning (absorbance) Real-time ratiometric measurements Well scanning
Microplate formats	Up to 3456-well plates, user-definable LVis Plate with 16 low-volume microspots (2 µL)
Microplate carrier	Robot compatible
Light sources	High energy xenon flash lamp Dedicated laser for AlphaScreen®/AlphaLISA®/AlphaPlex™ Dedicated laser for TRF and TR-FRET
Detectors	Two matched pairs of photomultiplier tubes (PMTs), dedicated to different detection modes CCD spectrometer
Wavelength selection	Optic Modules: up to six application-specific modules (includes all filters, dichroics, etc., for an application) For top and bottom reading UV/vis absorbance spectrometer: full spectrum or up to 8 distinct wavelengths in < 1 sec / well
Optical path	Top and bottom: free-air optical light path guided by motor-driven mirrors and dichroics
Z-adjustment	Automatic focal height adjustment (0.1 mm resolution)

Spectral range	**Filters**	230 - 750 nm or 230 - 900 nm for FI, FP 230 - 750 nm for LUM 230 - 900 nm for TRF
	Spectrometer	220 - 1000 nm for ABS; wavelength precision: ≤ 0.5 nm
Sensitivity	**FI** (top)	< 0.15 pM FITC (384sv black, 20 µL) < 0.5 pM FITC (1536 black, 8 µL)
	FI (bottom)	< 1.0 pM FITC (384g black, 50 µL)
	FI dynamic range	8 decades in a single measurement
	FP	< 0.5 mP SD at 1 nM FITC (384sv black, 20 µL) < 1.5 mP SD at 1 nM FITC (1536 black, 8 µL)
	TRF with laser	< 5 fM europium (384, 80 µL) < 15 fM europium (1536, 8 µL)
	HTRF® (black and white microplates)	Reader Control Kit (Eu) after 18h (384sv, 20 µL) Delta F > 1100 % (High Calibrator) Delta F > 25 % (Low Calibrator)
	LUM	< 0.4 pM (< 8 amol/well ATP, 384sv white, 20 µL)
	LUM dynamic range	8 decades in a single measurement
	AlphaScreen® with laser	< 5 pM (< 100 amol/well P-Tyr-100, 384sv white, 20 µL)*
	ABS with spectrometer	Selectable spectral resolution: 1, 2, 5, and 10 nm OD range: 0 to 4 OD; photometric resolution: 0.001 OD Accuracy: < 1% at 2 OD Precision: < 0.5% at 1 OD and < 0.8% at 2 OD Linearity: ≤ 0.8% at 2.0 OD
Read times	**Flying mode** (1 flash)	14 sec (384) 27 sec (1536)
	10 flashes	38 sec (384) 1 min 52 sec (1536)
	50 flashes	1 min 29 sec (384) 5 min 18 sec (1536)

Reagent injection	Up to 2 built-in reagent injectors Injection at measurement position (6 to 384-well) Individual injection volumes for each well (3 to 500 µL, optionally up to 2 mL) Variable injection speed up to 420 µL/sec Up to four injection events per well Reagent back flushing
Shaking	Linear, orbital, and double-orbital with user-definable time and speed
Integrated barcode reader	Up to three integrated barcode readers
Incubation	+5°C above ambient up to 45°C. The upper heating plate of the incubation chamber operates at +0.5°C than the lower plate. This prevents condensation build-up on the lid or sealer. With Advanced Assay Stability (AAS) system: from 18 - 45°C. Humidity sensor.
Software	Multi-user Reader Control and MARS data analysis software included FDA 21 CFR Part 11 compliant
Dimensions	Width: 45 cm, depth: 51 cm, height: 47 cm; weight: 49 kg With AAS system: width: 48 cm, depth: 68 cm, height: 47 cm; weight: 62 kg
	Accessories
Stacker	Plate handler for up to 50 microplates - continuous loading feature
THERMOstar	Microplate incubator and shaker
LVis Plate	Microplate designed to measure 16 low-volume (2 µL) samples and standard cuvettes. Incorporates NIST-traceable filters and holmium oxide standards for instrument performance test. Sensitivity: 2 ng/µL dsDNA
Upgrades	Please contact your local representative for upgrades including options such as detection modes, reagent injectors, etc.

* Limit of detection: < 100 amol of biotinylated and phosphorylated polypeptide (P-Tyr-100 assay kit, Revvity, #6760620C), measured in white 384 small volume microplates

Limit of detection (sensitivity) was calculated according to the IUPAC standard: $3 \times (SD_{blank}) / slope$
Specifications are subject to change without notice.
All mentioned products are for research use only.

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