Invitrogen™
life technologies

Enhance your protein profile

ZOOM® IEF Fractionator speeds and simplifies biomarker discovery

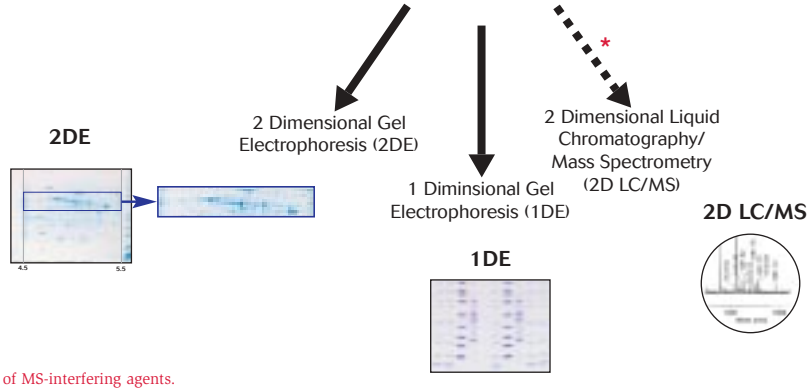
- **Solution phase IEF**–Allows easier detection of low abundance proteins and increases dynamic range of detection
- **Streamlines sample preparation**–Simple set up with all components ready to use
- **Provides superior results**–Reproducible separations over a broad pH range

Invitrogen
life technologies

Solution phase IEF for proteomics analysis

The ZOOM® IEF Fractionator is designed to fractionate samples by isoelectric point prior to 2DE, 1DE, or 2D LC/MS analysis (Figure 1). This method provides a simple, convenient, and reliable means to reduce sample complexity and enrich low abundance proteins. The ZOOM® IEF Fractionator uses ready-to-use immobilized buffer disks to fractionate samples into five pI ranges, at a scale and cost accessible to any lab.

Figure 1 - ZOOM® IEF fractionation



* Requires removal of MS-interfering agents.

Biomarker discovery made easy

Successful biomarker discovery through protein profiling can be a challenge to researchers faced with the task of identifying low abundance proteins in a complex sample. The ZOOM® IEF Fractionator rapidly concentrates proteins into zones of isoelectric point so that higher loads of rare proteins are ready for further analysis. Even with powerful techniques, such as two-dimensional electrophoresis (2DE), one-dimensional electrophoresis (1DE), and 2D Liquid Chromatography/Mass Spectrometry (2D LC/MS), reducing the complexity of the sample is often necessary for optimal detection efficiency. The convenience of the ZOOM® IEF Fractionator makes it easy and fast to perform this initial fractionation at a price any lab can afford.

The ZOOM® IEF Fractionator (Figure 2) combines reproducibility with user-friendly design. You get all the capabilities you require in a convenient, accessible format:

- Solution phase isolectric focusing (IEF)
- Small volume capability (670 µl of sample/chamber)
- Easy set up and sample recovery
- Integrated buffer chambers
- Removable electrode assembly
- Built-in safety features

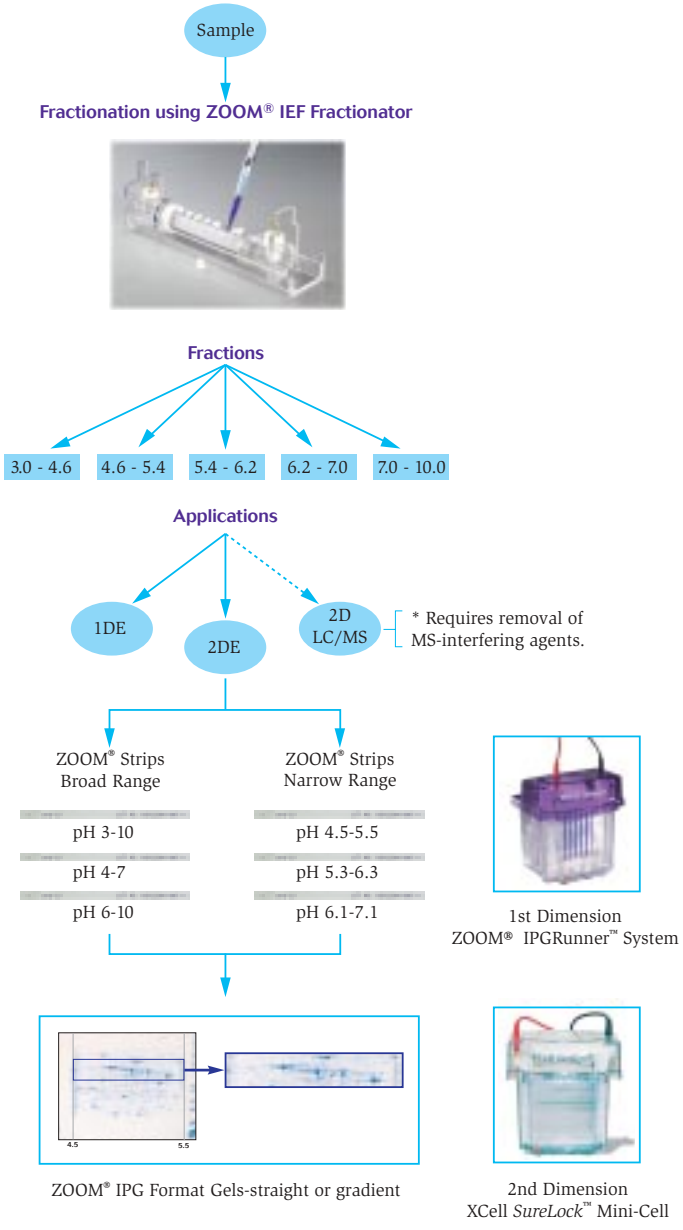
Advantages of ZOOM® IEF fractionation

Fractionation of proteins, especially highly complex samples like cell or tissue lysates, with the ZOOM® IEF Fractionator:

- Allows loading of increased amounts of proteins on IPG strips
- Results in high protein resolution and enhanced detection of low abundance proteins
- Increases sensitivity of detection, resulting in increased dynamic range of detection
- Reduces precipitation/aggregation artifacts of samples at high protein loads during 2DE

Figure 2 - ZOOM® IEF fractionation: application for 2DE

The flow path for fractionation of rat liver lysate using the ZOOM® IEF Fractionator. In addition, the flow path details the processing of fractionated sample by 2DE.



Invitrogen
life technologies

ZOOM® Disks maximize reliability

The ZOOM® IEF Fractionator's unique immobilized buffer disks—ZOOM® Disks (Figures 3, 4, and 5)—are convenient to use and ensure consistent fractionation. ZOOM® Disks are provided at pH values designed to separate typical proteomes into fractions having similar numbers of proteins based on genome analysis. The pre-cast disks are gel-based and disposable, designed for single use to minimize any chance of cross contamination. Pre-marked with the pH of the separation matrix, they are easy to handle and load with no preparation necessary.

ZOOM® Disks of specific pH ranging from pH 3 to pH 10 are available, allowing the fractionation of your sample into five equal fractions. Simply place each disk between the sample chambers in the ZOOM® IEF Fractionator to allow separation in a specific pH range. For example, if you want to fractionate proteins between pH 4.6 and 5.4, connect two sample chambers separated by ZOOM® Disks of pH 4.6 and pH 5.4. Sample fractions from the ZOOM® IEF Fractionator can be analyzed further by focusing on three broad range or three narrow range (single pH gradient) ZOOM® Strips (*i.e.*, pH 4-7 or 4.5-5.5, respectively, for analyzing the 4.6-5.4 fraction mentioned above).

Figure 3 - ZOOM® Disks

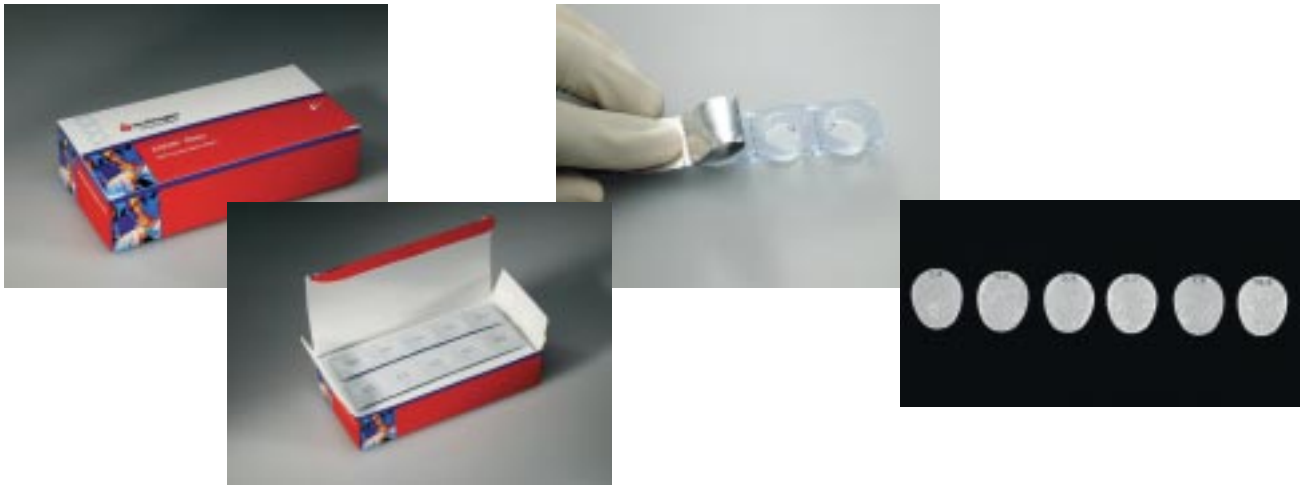


Figure 4 - ZOOM® IEF Fractionator components

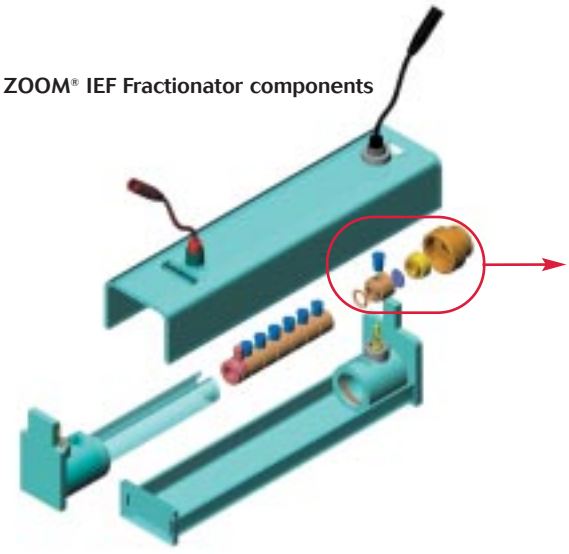
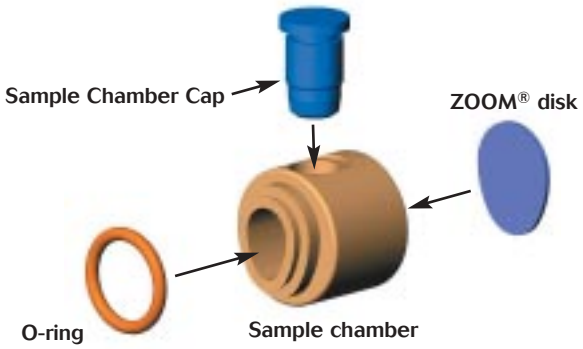


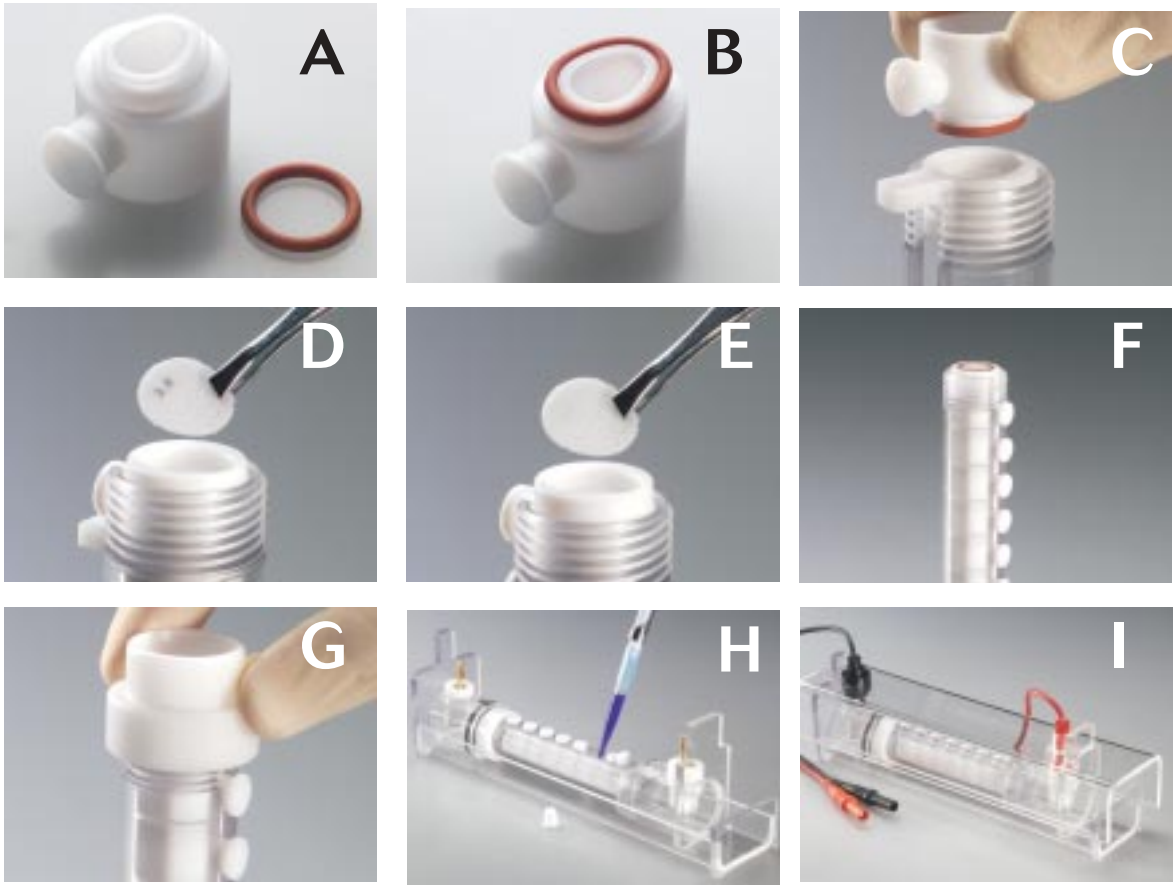
Figure 5 - Sample chamber assembly



Simple set up, easy run

The ZOOM® IEF Fractionator is designed for ease of use. First, set up the sample chambers (Figures 6 A through E) by inserting the pre-labeled ZOOM® Disks. Next, assemble the unit to form the buffer chambers and fill them with the appropriate buffers and samples (Figures 6 F through H). When you are ready to run, insert the lid (Figure 6I), and connect the electrodes to the power supply. Following a three-hour IEF run, the proteins are focused into five fractions. You'll have great spot resolution when the samples are processed further by 2DE, 1DE, or 2D LC/MS.

Figure 6 - ZOOM® IEF Fractionator sample chamber and unit set up



A. Insert Sample Chamber Cap into each chamber
B. Insert Sample Chamber O-ring in the front groove of each chamber
C. Insert anode end sealer into chamber assembly tube, then the first assembled sample chamber
D. Insert the appropriate ZOOM® Disk
E. Repeat steps to assemble the remaining sample chambers and disk
F. Complete process with the Cathode End Sealer
G. Screw in the Cathode End Screw Cap
H. Complete final assembly, add the samples and buffers, and insert the lid
I. Insert the lid and connect the electrodes to the power supply

Clear, reproducible results

The ZOOM® IEF Fractionator provides the reliability you need for successful biomarker discovery in downstream applications. To demonstrate this, rat liver lysate (RLL) was fractionated using the ZOOM® IEF Fractionator, followed by analysis of the fractions using broad range (pH 3-10, 4-7, and 6-10) and narrow range (pH 4.5-5.5) ZOOM® Strips. Figure 7a shows that the ZOOM® IEF Fractionator System can efficiently separate a complex proteome, such as rat liver tissue, into five well defined fractions based on pH. The fractionation reduces the sample's complexity while increasing the concentration of the fractionated proteins. Figures 7b demonstrates the improved ability to detect low abundance proteins in the fractionated sample that was applied to the narrow range ZOOM® Strip compared to the complex, unfractionated rat liver lysate protein sample (Figure 7c).

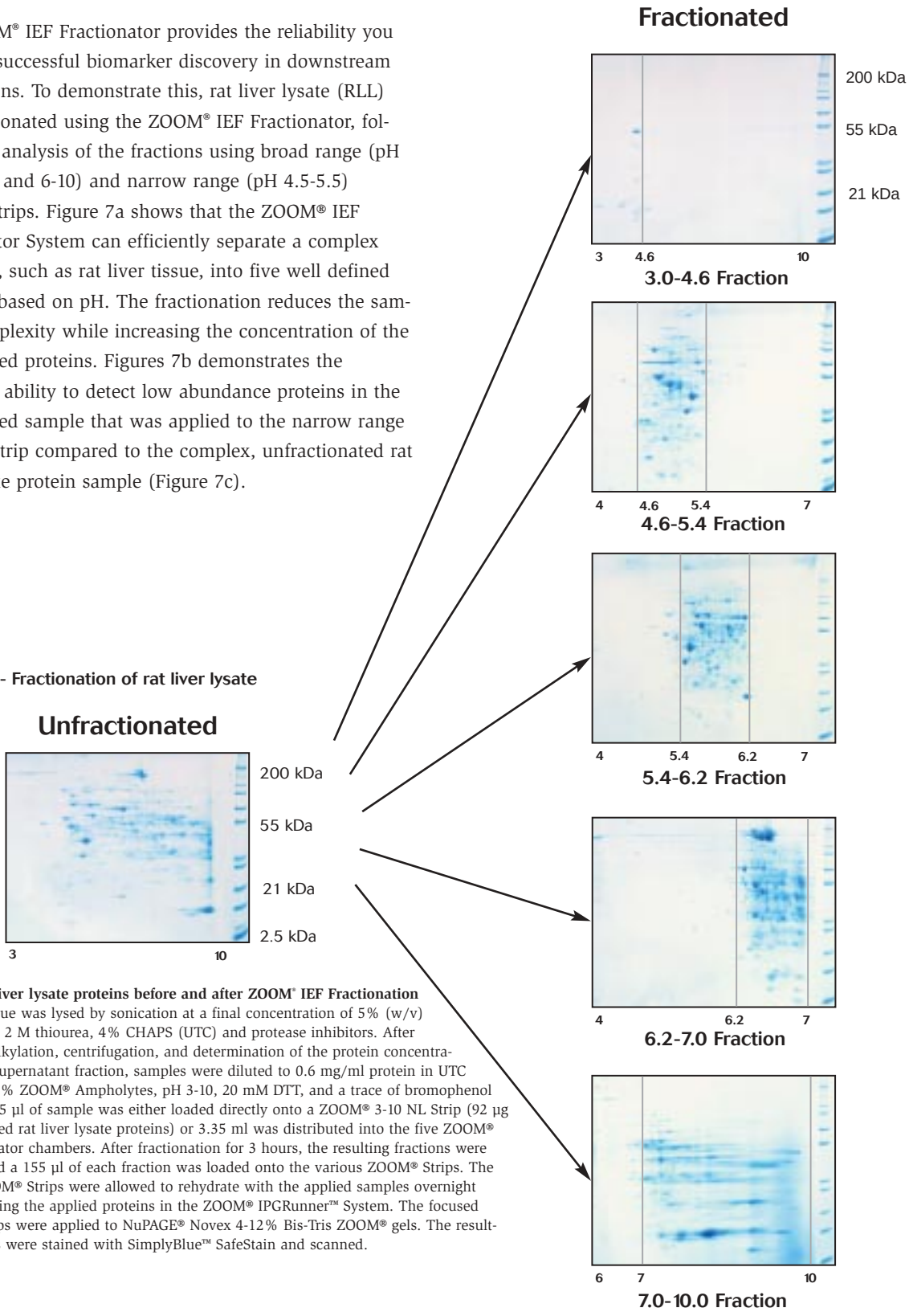
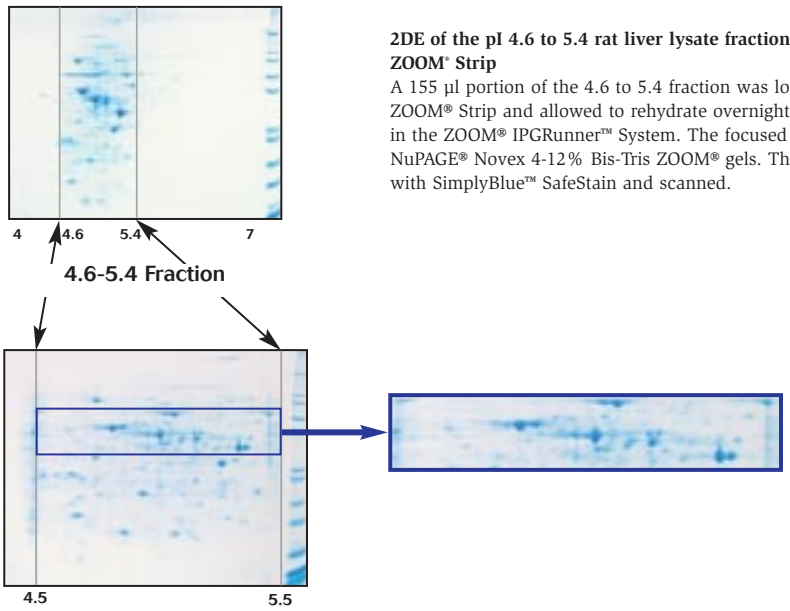


Figure 7a - Fractionation of rat liver lysate

2DE of rat liver lysate proteins before and after ZOOM® IEF Fractionation
Rat liver tissue was lysed by sonication at a final concentration of 5% (w/v) in 7 M urea, 2 M thiourea, 4% CHAPS (UTC) and protease inhibitors. After reduction, alkylation, centrifugation, and determination of the protein concentration of the supernatant fraction, samples were diluted to 0.6 mg/ml protein in UTC containing 1% ZOOM® Ampholytes, pH 3-10, 20 mM DTT, and a trace of bromophenol blue dye. 155 µl of sample was either loaded directly onto a ZOOM® 3-10 NL Strip (92 µg unfractionated rat liver lysate proteins) or 3.35 ml was distributed into the five ZOOM® IEF Fractionator chambers. After fractionation for 3 hours, the resulting fractions were collected and a 155 µl of each fraction was loaded onto the various ZOOM® Strips. The various ZOOM® Strips were allowed to rehydrate with the applied samples overnight before focusing the applied proteins in the ZOOM® IPGRunner™ System. The focused ZOOM® Strips were applied to NuPAGE® Novex 4-12% Bis-Tris ZOOM® gels. The resulting 2DE gels were stained with SimplyBlue™ SafeStain and scanned.

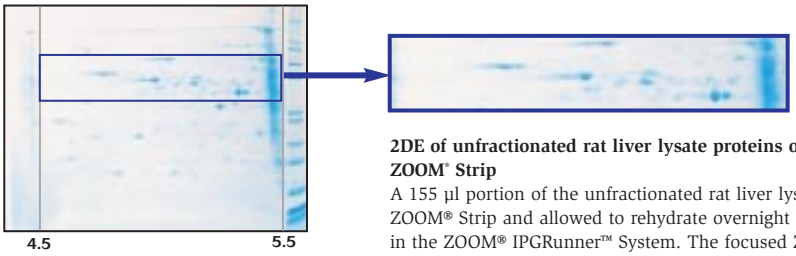
Clear, reproducible results, *continued*

Figure 7b - Fractionated rat liver lysate proteins



2DE of the pI 4.6 to 5.4 rat liver lysate fraction on a pH 4.5-5.5 narrow range ZOOM® Strip
A 155 µl portion of the 4.6 to 5.4 fraction was loaded on a pH 4.5-5.5 narrow range ZOOM® Strip and allowed to rehydrate overnight before focusing the applied proteins in the ZOOM® IPGRunner™ System. The focused ZOOM® Strips were applied to NuPAGE® Novex 4-12% Bis-Tris ZOOM® gels. The resulting 2DE gels were stained with SimplyBlue™ SafeStain and scanned.

Figure 7c - Unfractionated rat liver lysate proteins



2DE of unfractionated rat liver lysate proteins on a pH 4.5 to 5.5 narrow range ZOOM® Strip
A 155 µl portion of the unfractionated rat liver lysate was loaded on a 4.5-5.5 ZOOM® Strip and allowed to rehydrate overnight before focusing the applied proteins in the ZOOM® IPGRunner™ System. The focused ZOOM® Strips were applied to NuPAGE® Novex 4-12% Bis-Tris ZOOM® gels. The resulting 2DE gels were stained with SimplyBlue™ SafeStain and scanned.

ZOOM® Strips and the ZOOM® IPGRunner™ System: more innovative tools for benchtop proteomics

Three narrow range (1.0 pH unit) ZOOM® Strips from Invitrogen are fully compatible with three corresponding ZOOM® IEF Fractions. You can use narrow or broad range ZOOM® Strips on the ZOOM® IPGRunner™ System. The ZOOM® IPGRunner™ System provides a quick and convenient way to perform IEF of proteins using IPG strips for 2DE. For more details on the ZOOM® IPGRunner™ System, visit **www.invitrogen.com/zoom**.

Take the first step to successful biomarker discovery

Increase the resolution and detection of your protein samples with the simplicity, speed, and savings that the ZOOM® IEF Fractionator can offer. Order yours today.

Product	Quantity	Cat. no.
ZOOM® Fractionator Combo Kit	1 kit*	ZF10001
ZOOM® IEF Fractionator	1 ea	ZF10002
ZOOM® Disk pH 3.0	10 disks/pack	ZD10030
ZOOM® Disk pH 4.6	10 disks/pack	ZD10046
ZOOM® Disk pH 5.4	10 disks/pack	ZD10054
ZOOM® Disk pH 6.2	10 disks/pack	ZD10062
ZOOM® Disk pH 7.0	10 disks/pack	ZD10070
ZOOM® Disk pH 10.0	10 disks/pack	ZD10010
ZOOM® Urea	1 kg	ZU10001
ZOOM® Thiourea	1 kg	ZT10002
ZOOM® CHAPS	0.5 g	ZC10003
ZOOM® Carrier Ampholytes pH 3-10	10 ml	ZM0021
Novex® IEF Anode Buffer (50X)	100 ml	LC5300
Novex® IEF Cathode Buffer (10X)	125 ml	LC5310

* Includes all items in the quantity listed: ZOOM® IEF Fractionator; one pack ZOOM® Disks of each pH; urea; thiourea; CHAPS; carrier ampholytes pH 3-10; anode and cathode buffers.

References:

1. Zuo, X. and Speicher, D.W. (2000) *Anal. Biochem.* **284:** 266-278.
2. Zuo, *et al.* (2001) *Electrophoresis* **22:** 1603-1615.
3. Zuo and Speicher (2002) *Proteomics* **2:** 58-68.
4. Zuo, X., Hembach, P., Echan, L., and Speicher, D.W. (2002) *Journal of Chromatgraphy B* **782:** 253-265.
5. Ali-Khan, N., Zuo, X., and Speicher, D.W. (2002) *Current Protocols in Protein Science* **22.1:** 1-19.
6. Zuo, X. and Speicher D.W. (2002) *Methods in Molecular Biology* (Humana Press, P. Cutler, ed.) In press.

Invitrogen™
life technologies

713-022039 042803 15M

Corporate headquarters:
1600 Faraday Avenue • Carlsbad, CA 92008 USA • Tel: 760 603 7200 • Fax: 760 602 6500 • Toll Free Tel: 800 955 6288 • E-mail: tech_service@invitrogen.com • www.invitrogen.com

European headquarters:
Invitrogen Ltd • Inchinnan Business Park • 3 Fountain Drive • Paisley PA4 9RF, UK • Tel: +44 (0) 141 814 6100 • Fax: +44 (0) 141 814 6260 • E-mail: eurotech@invitrogen.com