invitrogen™

by life technologies™

ZOOM® IEF Fractionator

For fractionating complex protein samples using solution phase IEF

Catalog. nos. ZF10001 and ZF10002

Rev. Date: 22 June 2010

Manual part no. 25-0536

MAN0000296

User Manual

Contents

Safety Information

Informational Symbols

The symbols used on the ZOOM® IEF Fractionator are explained below:

Used on the Lid and in the manual to indicate an area where a potential shock hazard may exist.

Used on the Lid to indicate a warning. Consult the manual to avoid possible personal injury or instrument damage.

WEEE (Waste Electrical and Electronic Equipment) symbol indicates that this product should not be disposed of in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of WEEE. Visit www.invitrogen.com/weee for collection and recycling options.

Safety

The ZOOM® IEF Fractionator requires an external DC power supply designed for electrophoresis applications. **This power supply must be isolated from the ground so that the DC output is floating**. The maximum electrical operating parameters are:

Maximum Voltage:	1,500 VDC*
Maximum Power:	3.5 Watts*

The ZOOM® IEF Fractionator is designed to meet EN61010-1 Safety Standards. This product is safe to use when operated in accordance with this instruction manual. If this unit is used or modified in a manner not specified in this manual, the protection afforded by the unit will be impaired. Alteration of this unit will:

- Void the warranty
- Void the EN61010-1 safety standard certification
- Create a potential safety hazard (may result in shock or death)

Invitrogen is not responsible for any injury or damage caused by use of this unit when operated for purposes for which it is not intended. All repairs and services should be performed by Invitrogen. There are no parts that can be serviced by the user.

The lid of the ZOOM® IEF Fractionator is designed such that if the lid is removed the electrical connection to the device will be broken, but an electrical shock hazard exists as long as the electrode cords are plugged into the power supply.

To prevent any serious injury or death that may result from an electric shock:

- **Do not attempt to use the cell without the lid**
- **Do not handle or wash the lid with the cables plugged into the power supply**

*The recommended levels for optimal performance are less than the maximum voltage/wattage given above, see page 18 for details on performing fractionation.

Sicherheits-Anweisungen

Symbolerklärung

Im folgenden Abschnitt sind die Gefahrenhinweissymbole auf dem ZOOM® IEF Fractionator erklärt:

Gefahr

Weist auf eine Zone hin, wo Gefährdung durch Stromschlag besteht.

Warnung

Lesen Sie die Bedienungsanleitung, um Verletzungen oder Beschädigung des Gerätes zu vermeiden.

WEEE

Sicherheits-Anweisungen

Die ZOOM® IEF Fractionator muss mit einer geerdeten Gleichstrom-Spannungsquelle betrieben werden, die speziell für die Elektrophorese entwickelt wurde. **Die Stromquelle darf nicht geerdet sein, damit die DC Ausgänge potenzialfrei sind.** Um eine Gefährdung des Benutzers auszuschließen, sollten folgende maximalen Laufbedingungen eingehalten werden:

Maximale Spannung:	1500 Volt
Maxi male Leistung:	3.5 Watt

Die ZOOM® IEF Fractionator entspricht den Sicherheits-Richtlinien EN 61010-1. Es besteht keinerlei Gefährdung für den Benutzer, wenn die Richtlinien der Gebrauchsanweisung eingehalten werden. Wird die Elektophorese-Zelle in einer Art und Weise benutzt bzw. modifiziert, die nicht in der Gebrauchsanleitung beschrieben ist, werden die Sicherheitsvorkehrungen zum Schutz des Betreibers beeinträchtigt. Bei unsachgemäßer Handhabung entfallen alle Garantieansprüche und Sicherheitszulassungen (EN61010-1). Ausserdem besteht die potentielle Gefahr eines Elektroschocks der zum Tod führen kann.

Invitrogen haftet nicht für Verletzungen oder Schäden jeglicher Art, wenn die Elektrophorese-Zelle für Anwendungen eingesetzt wird, für die die Zelle nicht entwickelt wurde. Alle Reparaturen und Wartungen müssen von Invitrogen oder einem von Invitrogen authorisierten Händler vorgenommen werden. Keine Teile des Geräts können vom Anwender gewartet werden.

Gefahr

Beim Entfernen des Kammerdeckels der ZOOM® IEF Fractionator wird der Stromfluss unterbrochen. Eine Gefährdung durch Stromschlag besteht aber noch solange, wie das Gerät an die Spannungsquelle angeschlossen ist. Um Schäden aufgrund eines Elektroschocks auszuschliessen, sollte niemals versucht werden, die Kammer ohne Deckel zu betreiben. Sie sollten bei bestehender Verbindung zur Spannungsquelle nicht mit dem Deckel hantieren oder ihn waschen. Die Benutzung der Zelle ohne einen für das Gerät vorgesehenen Deckel ist verboten.

Instructions de sécurité

Signification des symboles

Les symboles utilisés sur l'appareil ZOOM® IEF Fractionator sont expliqués ci-dessous:

Précaution

Sur le couvercle de l'appareil ZOOM® IEF Fractionator et dans le manuel pour signaler les zones où un risque potentiel de choc électrique peut exister.

Danger

Sur l'appareil ZOOM® IEF Fractionator et dans le manuel pour signaler un danger. Veuillez consulter le mode d'emploi pour éviter tout risque potentiel pour l'utilisateur ou pour l'équipement.

WEEE

Instructions de sécurité

L'appareil ZOOM® IEF Fractionator doit être uniquement utilisé avec une alimentation en courant continu DC externe et spécialement conçue pour l'électrophorèse. **Cette alimentation doit être avec séparation galvanique de façon que la sortie DC du secondaire aie un potentiel électrique flottant**. Le domaine d'utilisation de l'appareil ZOOM® IEF Fractionator est limité par les paramètres de fonctionnement suivants:

Tension maximum:	1500 V continu
Puissance maximum:	3.5 W

L'appareil ZOOM® IEF Fractionator satisfait aux normes de sécurité EN 61010-1. La sécurité est assurée dans le cas d'une utilisation conforme au manuel d'utilisation. Dans le cas où la cellule serait modifiée ou utilisée de manière non spécifiée dans le manuel d'utilisation, la sécurité de l'utilisateur pourrait être remise en cause. En cas d'utilisation non conforme toutes les garanties ainsi que les certifications de conformité aux normes de sécurité sont abrogées.

La responsabilité de Invitrogen ne peut être mise en cause en cas d'accident et de dommages causés par une utilisation non conforme de la Cellule d'Electrophorèse. Toute intervention de service ou de réparation doit être uniquement effectuée par Invitrogen ou par un agent agrée par Invitrogen. Aucune partie ne peut être réparée par l'utilisateur.

Précaution

L'appareil ZOOM® IEF Fractionator est conçu de façon a ce que lorsque le couvercle de la cuve est ouvert, la connexion électrique avec l'unité est interrompue, mais un risque de choc électrique persiste tant que les électrodes sont branchées.

- Ne pas essayer de faire fonctionner l'appareil sans son couvercle.
- Ne pas toucher ou nettoyer le couvercle lorsque les câbles sont branchés.

Kit Contents

Products

This manual is supplied with the following products:

Product	Catalog no.
ZOOM® IEF Fractionator Combo Kit	ZF10001
ZOOM® IEF Fractionator	ZF10002

Shipping and Storage

The ZOOM® IEF Fractionator is shipped at room temperature. Store at room temperature.

The ZOOM® IEF Fractionator Combo Kit includes the ZOOM® IEF Fractionator, shipped at room temperature, **and** a Combo box containing ZOOM® Disks, ZOOM® Carrier Ampholytes, pH 3–10, and Novex® IEF Cathode Buffer, which is shipped on blue ice. Upon receipt, store the ZOOM® IEF Fractionator box at room temperature and the Combo box at 4°C.

ZOOM® IEF Fractionator Components

The ZOOM® IEF Fractionator Kit and the Combo Kit both include the assembled ZOOM® IEF Fractionator, one bag of Sample Chamber Caps, and two boxes of Spares (spare parts).

The components of the ZOOM® IEF Fractionator are listed below. For more details on each component, see page 6.

Product	Components	Quantity
ZOOM® IEF Fractionator	Chamber Assembly Tube with Anode Reservoir	1
	Spill Trough with Cathode Reservoir	1
	Lid	1
	Sample Chambers	7
	Sample Chamber O-ring Seals, red	10
	Sample Chamber Caps with O-rings	7
	Cathode End Sealer	1
	Anode End Sealer	1
	Cathode End Screw Cap	1
	Spacers, black	8
	Spares Box 1	
	• Sample Chamber O-ring Seals	8
	• Sample Chamber Caps with O-rings	7
	Spares Box 2	
	• Spacers, black	8
	• Cathode Chamber Seals	2

Continued on next page

ZOOM® IEF Fractionator Combo Kit—Additional Components

The ZOOM® IEF Fractionator Combo Kit includes the ZOOM® IEF Fractionator (see previous page) and a Combo box containing one pack of ZOOM® Disks of each pH and ZOOM® proteomic-grade reagents.

The Combo box components are listed below. Sufficient disks are supplied to perform 10 fractionations using a set of six disks (pH 3.0–10.0).

Product	Components	Quantity
ZOOM® IEF Fractionator Combo Kit—Combo Box	ZOOM® Disk pH 3.0	1 pack of 10
	ZOOM® Disk pH 4.6	1 pack of 10
	ZOOM® Disk pH 5.4	1 pack of 10
	ZOOM® Disk pH 6.2	1 pack of 10
	ZOOM® Disk pH 7.0	1 pack of 10
	ZOOM® Disk pH 10.0	1 pack of 10
	ZOOM® Urea	1 kg
	ZOOM® Thiourea	0.5 kg
	ZOOM® CHAPS	5 g
	Novex® IEF Anode Buffer (50X)	100 mL
	Novex® IEF Cathode Buffer pH 3–10 (10X)	2 × 125 mL
	ZOOM® Carrier Ampholytes, pH 3–10	10 mL

Intended Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use

Product Specifications

ZOOM® IEF Fractionator Specifications

Dimensions:	28 cm × 6.5 cm × 6.5 cm
Anode Buffer Chamber Capacity:	18 mL
Cathode Buffer Chamber Capacity:	18 mL
Material:	Polycarbonate/Acrylic/Delrin
Black O-ring Material:	Viton
Red O-ring Material:	Silicon rubber
Sample Chamber Material:	Teflon
Sample Chamber Capacity:	750 µL
Electrode Wire:	Platinum (0.010″diameter)
Temperature Limit:	5–40°C

The ZOOM® IEF Fractionator is impervious to alcohol, but not compatible with chlorinated hydrocarbons (e.g. chloroform), aromatic hydrocarbons (e.g. toluene, benzene) or acetone.

For more details on each part of the ZOOM® IEF Fractionator, see page 6.

CE

The ZOOM® IEF Fractionator is CE certified and complies with the European Community Safety requirements. Operation of the ZOOM® IEF Fractionator is subject to the conditions described in the manual (see page 18).

The protection provided by the equipment may be impaired if the equipment is used in a manner not specified by Invitrogen.

Maintenance

Wash the ZOOM® IEF Fractionator with a mild detergent and rinse with deionized water after each use. For more details on cleaning the ZOOM® IEF Fractionator, see page 27. **Do not wash the lid with the cables plugged into the power supply.**

Note: There are no serviceable parts for this product. Do not attempt to perform any servicing to the ZOOM® IEF Fractionator.

Introduction

Product Description

About the Product

The ZOOM® IEF Fractionator provides a simple and convenient method to reproducibly fractionate cell and tissue lysates into well-resolved fractions on the basis of isoelectric point (pI) using solution phase isoelectric focusing (IEF).

The major components of the ZOOM® IEF Fractionator System are:

- ZOOM® IEF Fractionator (see next page)
- ZOOM® Disks (see page 4)

For more details on these components, see pages 2–4.

Fractionation using solution phase IEF in small volumes combined with other downstream methods for protein profiling such as 2D gel electrophoresis on narrow pH range strips results in obtaining detailed protein profiles of various proteomes (Zuo *et al.*, 2001; Zuo & Speicher, 2002).

Solution Phase IEF

Native protein fractionation using solution phase IEF was pioneered by Bier *et al.,* (1979) using ampholytes to form pH gradients in a horizontal, preparative apparatus with sample chamber volumes of 60 mL. Improvements to this method were performed using a 30–100 mL sample chamber volume preparative apparatus that used segmented chambers formed by immobilized pH membranes (Faupel *et al.*, 1987). These large-scale, preparative apparatus performed IEF in solution phase and focused the proteins of interest into specific sample chambers based on the pI of proteins.

Zuo and Speicher (2000) first described the fractionation of complex proteomes into complete sets of proteins under denaturing conditions using solution phase IEF in small (~0.5 mL) sample volumes. The ZOOM® IEF Fractionator is based on the method of Zuo and Speicher (2000).

Purpose of this Manual

This manual provides the following information:

- Overview of the ZOOM® IEF Fractionator
- Sample preparation guidelines
- Using the ZOOM® IEF Fractionator for IEF
- Troubleshooting

Note: Detailed protocols for sample preparation and processing fractions obtained from the ZOOM® IEF Fractionator for downstream applications, except two-dimensional gel electrophoresis, are not included in this manual.

Continued on next page

Product Description, Continued

Advantages of Fractionation

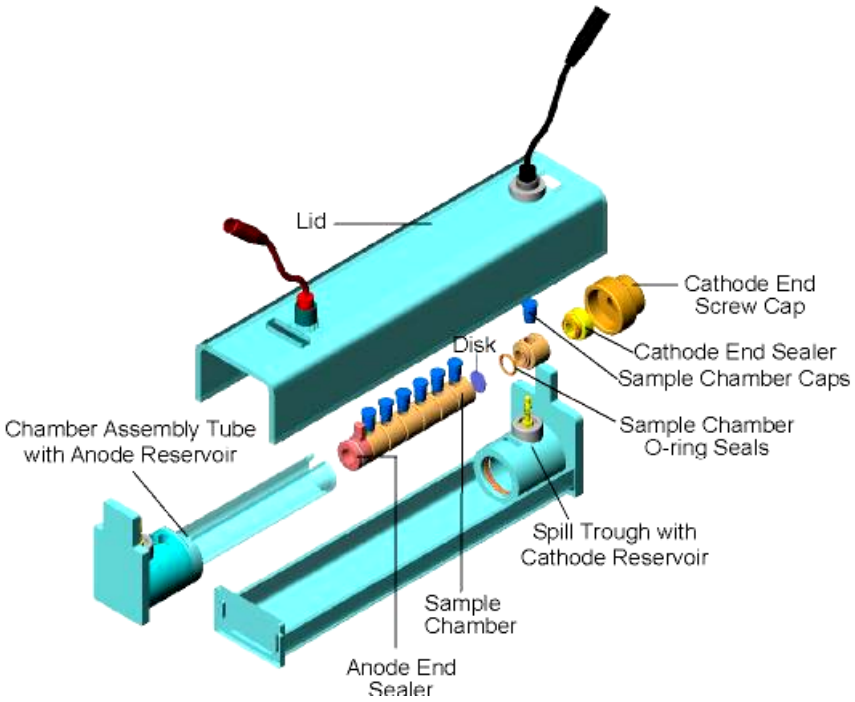
Performing fractionation of complex samples (cell and tissue lysate) using the ZOOM® IEF Fractionator offers the following advantages:

- Allows loading of increased amounts of protein for downstream applications
- Reduces sample complexity
- Results in high resolution and identification of low abundance proteins
- Increases the dynamic range of detection by increasing the concentration of proteins
- Reduces precipitation/aggregation artifacts of samples at high protein loads during 2D gel electrophoresis

ZOOM® IEF Fractionator

The ZOOM® IEF Fractionator is used to fractionate complex samples using solution phase IEF. The design of the ZOOM® IEF Fractionator is based on the technique developed by Zuo and Speicher (2000) . This basic design was modified to improve the ease, speed, and efficiency of sample application and recovery.

The figure below shows the various components of the ZOOM® IEF Fractionator. See page 6 for more details on each part.



Continued on next page

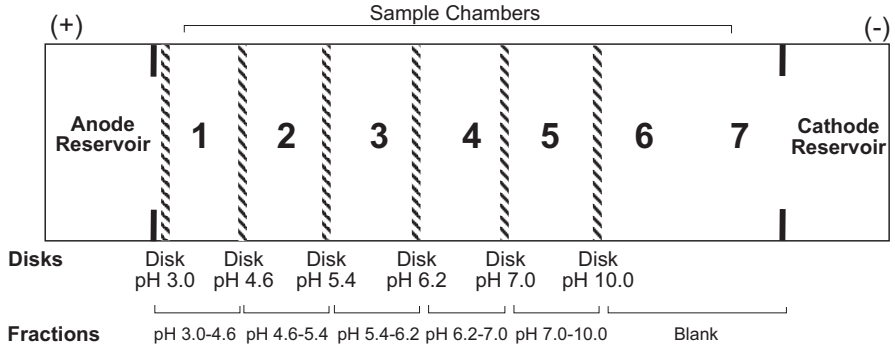
Fractionator Configurations

Fractionation in the ZOOM® IEF Fractionator is performed using a series of Sample Chambers connected in tandem and separated by thin membranes (ZOOM® Disks, see next page) containing covalently attached buffers of defined pH. The protein sample is loaded into multiple Sample Chambers separated by these disks or a combination of disks and spacers and subjected to solution phase IEF for 3 hours. At the end of solution phase IEF, the protein sample is separated into well-resolved fractions based on the pI of each protein (see figures below).

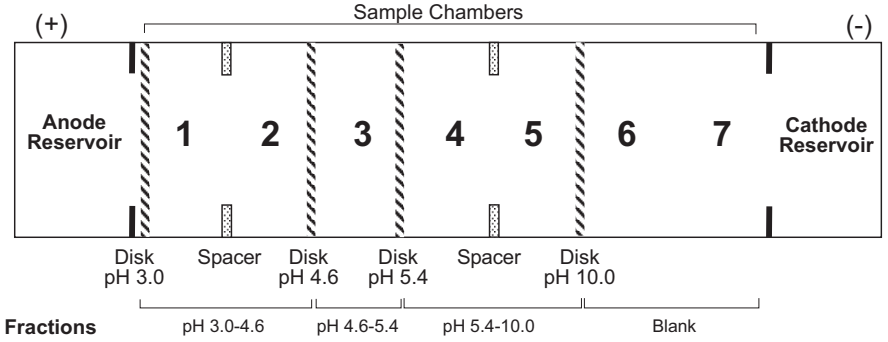
You can assemble the tube with various combinations of disks and spacers to cover a standard pH range (pH 3.0–10.0; see example below), an extended pH range (pH 3.0–12.0), or narrow pH ranges (e.g., pH 4.6–5.4, pH 9.1–12; see example below). See page 19 for additional information about configuring the fractionator.

The fractions from the ZOOM® IEF Fractionator are suitable for analysis using various downstream applications (see next page). Fractionated samples can be further separated on a series of overlapping narrow pH range ZOOM® Strips (IPG strips), resulting in the effective fractionation of a complex proteome.

Configuration for fractionation from pH 3.0 to pH 10.0



Configuration for fractionation from pH 4.6 to pH 5.4



Continued on next page

Product Description, Continued

Features of the ZOOM® IEF Fractionator

Important features of the ZOOM® IEF Fractionator are listed below:

- Unique, user-friendly design for easy set-up and sample recovery
- Ability to perform small volume (650 µL/chamber) solution phase IEF to prevent sample dilution
- Tight-sealing chambers to ensure reproducible, leak-free fractionation
- Built-in safety features to enhance user safety
- Removable electrode assembly for easy cleaning

ZOOM® Disks

ZOOM® Disks are polyacrylamide gels containing covalently attached buffers of defined pH cast in a fritted polyethylene support. The ZOOM® Disks are disposable and the pH is marked on each disk for easy identification.

The ZOOM® Disks are supplied ready to use. Each ZOOM® Disk box contains 10 disks of the same pH individually packaged in foil pouches with 1 mL of packaging buffer. **Caution:** The packaging buffer contains 0.02% sodium azide and residual acrylamide monomer. Wear gloves at all times while handling ZOOM® Disks.

Each ZOOM® Disk is inserted between Sample Chambers to create chambers that will allow separation of proteins in specific pH ranges (see figure on previous page). For example, inserting ZOOM® Disks pH 3.0 and pH 4.6 creates a chamber for fractionating proteins between pH 3.0–4.6.

Downstream Applications

The fractions of the ZOOM® IEF Fractionator are suitable for analysis using various downstream applications for protein profiling such as:

- Two-dimensional (2D) Gel Electrophoresis (see next page)
- One-dimensional Electrophoresis (Zuo *et al.*, 2002)
- Two-dimensional Liquid Chromatography/Mass Spectrometry (2D LC/MS)

Continued on next page

Two-Dimensional (2D) Gel Electrophoresis

Two-dimensional (2D) gel electrophoresis is a powerful and sensitive technique for separating and analyzing protein mixtures from biological samples. The steps of 2D gel electrophoresis are described below:

1. First dimension separation of proteins using isoelectric focusing.

   Proteins are separated based on their isoelectric point or pI. The proteins are applied to polyacrylamide gels containing ampholytes or immobilized pH gradient (IPG) strips containing a fixed pH gradient. As the protein sample containing a mixture of different proteins migrates through the pH gradient, individual proteins are immobilized in the pH gradient as they reach their isoelectric point.

2. Second dimension separation of proteins using SDS-PAGE.

   Proteins are separated based on their molecular weight using denaturing polyacrylamide gel electrophoresis.

3. Detection of proteins on the gel.

   The second dimension gel is stained using an appropriate staining procedure to visualize the separated proteins as spots on the gel or the proteins from the gel are blotted onto membranes.

4. Analysis of protein spots.

   Protein spots are excised from the SDS gel or membranes and subjected to further analyses such as mass spectrometry or chemical microsequencing to facilitate protein identification by comparing to various databases.

Description of Parts

Introduction

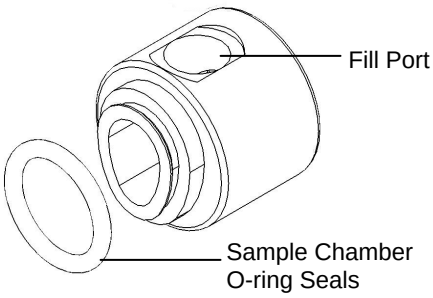
The various parts of the ZOOM® IEF Fractionator are described in this section. For details on assembling the various parts, see page 18.

Note: The parts of the ZOOM® IEF Fractionator are supplied assembled in the Chamber Assembly Tube. You need to disassemble the fractionator and wash various parts of the fractionator before use as described on page 18.

Sample Chamber and O-ring Seal

The Sample Chambers are designed to hold 650 µL of protein sample. The Sample Chamber O-ring Seal (O-ring) is placed on the groove and is used for creating a tight seal between the disk and Sample Chamber. Seven Sample Chambers and ten Sample Chamber O-ring Seals are included with the ZOOM® IEF Fractionator. The Spares Box contains 8 additional Sample Chamber O-ring Seals.

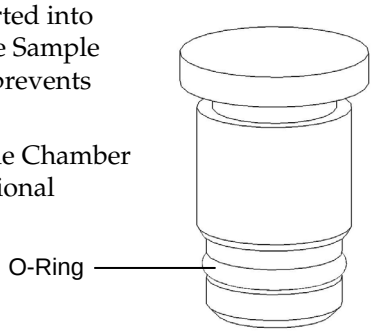
A fill-port is provided on top of the Sample Chamber to conveniently add and remove the sample and insert the Sample Chamber Cap.



Sample Chamber Caps

The Sample Chamber Cap with O-Ring is inserted into the fill port on top of the Sample Chamber. The Sample Chamber Cap seals the Sample Chamber and prevents any leakage of samples.

The ZOOM® IEF Fractionator includes 7 Sample Chamber with O-Rings. The Spares Box includes 7 additional Sample Chamber Caps with O-Rings.



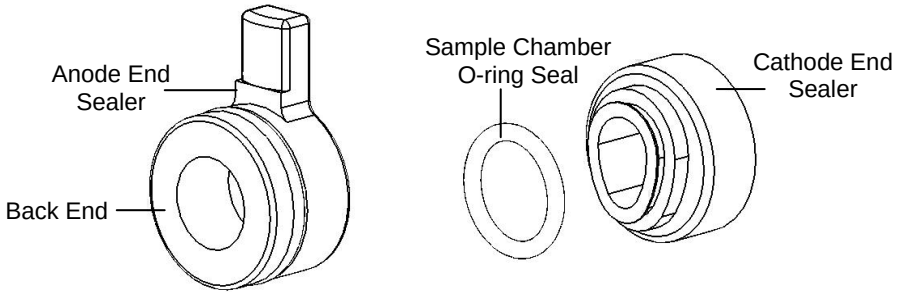
Continued on next page

Anode and Cathode End Sealers

The Anode End Sealer is inserted first at the anode end of the Chamber Assembly Tube. The protrusion on top of the Anode End Sealer assists in pushing out the Sample Chambers at the end of fractionation. A black friction O-ring is attached to the Anode End Sealer to allow you to easily assemble the sealer and Sample Chambers into the Chamber Assembly Tube.

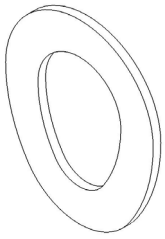
The Cathode End Sealer is inserted at the cathode end of the Chamber Assembly Tube after adding all Sample Chambers to the Chamber Assembly Tube. The Cathode End Sealer helps to seal the Sample Chambers in position in the Chamber Assembly Tube. A Sample Chamber O-ring Seal is placed on the groove of the Cathode End Sealer to ensure reproducible sealing.

The ZOOM® IEF Fractionator includes 1 Anode End Sealer and 1 Cathode End Sealer.



Spacer

A Spacer may be inserted between two Sample Chambers instead of a ZOOM® Disk. A Spacer creates one large continuous chamber from adjoining chambers. If you are using fewer than six ZOOM® Disks in a fractionator assembly, use spacers to create an assembly of the proper length. Spacers are not needed if you are using at least six ZOOM® Disks.



The ZOOM® IEF Fractionator includes eight Spacers. An additional eight Spacers are included in the Spares Box.

Continued on next page

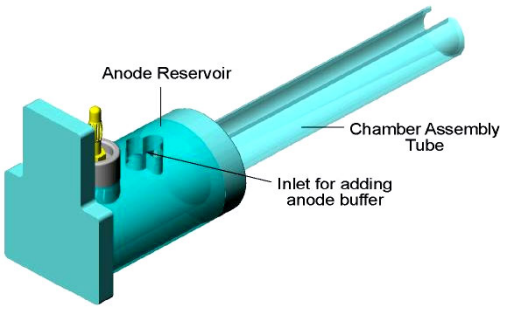
Description of Parts, Continued

Chamber Assembly Tube

The Chamber Assembly Tube is designed to hold seven Sample Chambers, up to eight ZOOM® Disks, an Anode End Sealer, and a Cathode End Sealer.

The Chamber Assembly Tube also contains the anode reservoir and the anode electrode assembly. The anode reservoir holds 18 mL anode buffer. An inlet is provided on the anode reservoir (designed as +) for adding anode buffer.

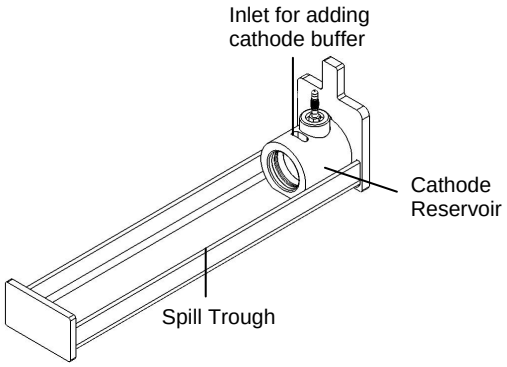
The Cathode End Screw Cap is attached at the end of the Chamber Assembly Tube to ensure reproducible sealing of the Sample Chambers and helps seal the Chamber Assembly Tube to the cathode reservoir for fractionation.



Spill Trough

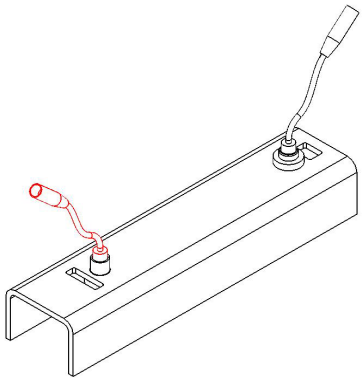
The Spill Trough includes the cathode electrode assembly, cathode reservoir, and a spill trough for safety.

The cathode reservoir holds 18 mL cathode buffer. An inlet is provided on the cathode reservoir (designed as -) for adding cathode buffer. The Chamber Assembly Tube is placed in the Spill Trough during fractionation.



Lid

The lid will fit onto the unit in only one orientation to prevent incorrect connection to the electrodes.



Experimental Overview

Experimental Outline

The table below outlines the experimental steps necessary to perform fractionation using the ZOOM® IEF Fractionator. For more details on each step, see indicated pages.

Step	Action	Page
1	Prepare protein samples.	11
2	Prepare anode and cathode buffers and assemble the Sample Chambers.	21
3	Assemble the Chamber Assembly Tube with Sample Chambers and disks.	21
4	Load samples into the Sample Chambers.	24
5	Add diluted anode buffer to the anode reservoir and diluted cathode buffer to the cathode reservoir.	24
6	Perform fractionation using the recommended parameters.	25
7	Remove Sample Chamber Caps from Sample Chambers and retrieve your fractionated samples.	26
8	Use fractionated protein samples for the downstream application of choice.	28

Materials Needed

- Power supply (see next page)
- Forceps
- Lysis Buffer
- 1 M Tris Base
- 2 M DTT
- N,N-Dimethylacrylamide (DMA) from Aldrich, Cat. no. 27413-5
- Protease Inhibitor tablets (Roche cat. no. 1873580)
- ZOOM® Focusing Buffers pH 3–7 and pH 7–12 (recommended) *or* ZOOM® Carrier Ampholytes (optional)
- ZOOM® 2D Protein Solubilizer (recommended), or other sample buffer
  Additional materials for use with ZOOM® 2D Protein Solubilizer:
  - Benzonase from Sigma, Cat. no. E-1014
  - Tributylphosphine (TBP) from Sigma, Cat. no. T-7567
  - 20% (w/v) SDS
  - **Cold** acetone
  - **Cold** (–20°C) 10% (w/v) TCA, 0.07% (v/v) β-mercaptoethanol in acetone (~1 mL/0.3 g tissue) for plant lysate
  - **Cold** (–20°C) 0.07% (v/v) β-mercaptoethanol in acetone (~20 mL) for plant lysate

Continued on next page

Power Supply Requirements

The ZOOM® IEF Fractionator is used with an external DC power supply designed for electrophoresis and IEF applications. This power supply must:

- Be isolated from the ground so that the DC output is floating
- Be able to operate at low current (<1 mA) required to perform IEF

Many power supplies automatically shut-off when the current drops below 1 mA. You will need a power supply capable of overriding the low current shut-off feature. Contact the manufacturer for more information on your power supply.

The electrical leads of the ZOOM® IEF Fractionator lid are recessed and may not fit into some power supply units. To allow connection of the ZOOM® IEF Fractionator power leads with certain power supplies, use Novex® Power Supply Adapters available separately (page 42) or contact Technical Support (see page 45).

Methods

Preparing Samples

Introduction

Proper sample preparation is key to the success of an IEF experiment. Various factors affect the design of a sample preparation protocol. General guidelines for sample preparation are provided in this section.

A recommended procedure for preparing samples using the ZOOM® 2D Protein Solubilizer Kit and ZOOM® Focusing Buffers is also provided in this section. An alternative procedure using reagents provided in the ZOOM® IEF Fractionator Combo Kit is provided on page 38.

For more information on sample preparation protocols, see published literature (Rabilloud, 1999; Rabilloud, 2000).

Note

The ZOOM® 2D Protein Solubilizer Kit, and ZOOM® Focusing Buffers pH 3–7 and 7–12 recommended in this section are available separately from Invitrogen. See ordering information on page 42.

Important

Due to the large variety of proteins present in different cells and tissues, it is not possible to have a sample preparation protocol that is suitable for all proteins. Based on the starting material and goal of the experiment, the sample preparation protocol needs to be determined empirically. The sample preparation conditions may also be optimized based on your initial results.

Review this section carefully to gain a better understanding of sample preparation requirements prior to preparing your sample.

Objectives

The major objectives of sample preparation are to:

- Completely solubilize proteins
- Denature proteins
- Reduce and alkylate disulfide bonds in proteins prior to IEF fractionation (Galvani *et al.*, 2001a; Galvani *et al.*, 2001b; Herbert *et al.*, 2001)
- Prevent protein modification and proteolysis
- Maintain proteins in solution during solution phase IEF

Continued on next page

Considerations for Sample Preparation

It is important to consider the following points for sample preparation to obtain the desired results using your sample:

- It is helpful to have some knowledge of your sample prior to sample preparation. Knowledge about your sample helps in determining the method of extraction, choice of sample buffer, and type of interfering components present in the sample.
- Contaminants such as salts, conductive ions, nucleic acids, and insoluble materials will affect IEF. Removal of these contaminants using dialysis, centrifugation, filtration, or precipitation improves the resolution. **Note:** Performing multiple steps to improve the sample quality may result in loss of some proteins.

Guidelines for Sample Preparation

- Prepare your protein sample at a suitable concentration using an appropriate sample buffer. We recommend using the ZOOM® 2D Protein Solubilizer Kit for sample preparation as described on page 14.
- Dilute the sample to a final concentration of 0.5 mg/mL.
- Avoid using iodoacetamide for alkylation of samples as iodoacetamide will create extremely high currents and poor fractionation results.
- Avoid repeated freezing and thawing of samples or heating samples above 30°C in the presence of urea (Righetti, 1983).
- Always maintain non-urea samples (samples without urea) on ice to minimize proteolysis.
- Avoid using a complex sample preparation strategy as it may result in protein loss.
- Remove any particulate material by high-speed centrifugation or filtration using 0.2 µm low-protein binding filter as the particulate material can clog the pores of ZOOM® Disks.
- Maintain the salt concentration of the sample at < 10 mM.

Amount of Protein

For sample fractionation, dilute the protein sample to a final concentration of 0.5–0.6 mg/mL as described on page 17. You will need 650 µL of sample for each Sample Chamber of the ZOOM® IEF Fractionator.

The ZOOM® IEF Fractionator is designed to reproducibly fractionate 1–2 mg of protein. **Note:** If you wish to fractionate >2 mg protein, you may need to optimize fractionation conditions.

Continued on next page

Sample Buffer

The first step in sample preparation is choosing and/or preparing a suitable sample buffer. Due to the large variety of proteins, there is no universal sample buffer. Based on the starting material and goal of the experiment, the sample buffer composition may be optimized.

An ideal sample buffer must maintain the proteins in solution during IEF and not have any effect on the pI of the protein. The sample buffer generally contains a denaturing agent (urea or urea/thiourea), solubilizing agent (non-ionic or zwitterionic detergent and ampholytes), and reducing agent (DTT).

The major components of the sample buffer and their functions are listed below. The recommended concentration for each component is also provided. Note the final concentration must be optimized based on the solubility of your proteins. Optimization is usually achieved by varying the concentrations of detergent, urea, ampholytes, and reducing agent.

Component	Function	Final Concentration
Urea Urea solutions are prepared fresh or stored frozen at -80°C. De-ionize urea solutions on a mixed-bed ion exchanger resin using manufacturer's recommendations. Thiourea is used to increase solubility of some proteins (Rabilloud, 1998)	Denatures and solubilizes proteins	8 M urea or 9 M urea in some cases For urea/thiourea solution, use 5-8 M urea with 2 M thiourea
Detergent Use non-ionic or zwitterionic detergents such as CHAPS, NP-40, CHAPSO, and sulfobetaines (SB3-10) (Chevallet *et al.*, 1998). Ionic detergents such as SDS are not recommended.	Solubilizes the proteins and helps to maintain the proteins in solution during rehydration and IEF	Total detergent concentration range is 0.5–5%
Reducing Agent Use DTT or DTE (dithioerythritol). β-mercaptoethanol is not recommended for reduction (Righetti *et al.*, 1982).	Cleaves the disulfide bonds in proteins and maintains cysteine, methionine, and tryptophan from oxidizing	DTT or DTE at 20-100 mM
Focusing Buffer We recommend using ZOOM® Focusing Buffer for all fractionations with the ZOOM® IEF Fractionator. An alternative protocol using ZOOM® Carrier Ampholytes is provided on page 40.	Helps solubilize proteins, maintain the pH gradient, and provide carrier ions for proteins	20 µL

Continued on next page

Materials Needed

- Protein sample (see page 12 for the amount of protein sample needed)
- Lysis Buffer
- 1 M Tris Base
- 2 M DTT
- N,N-Dimethylacrylamide (DMA) from Aldrich, Cat. no. 27413-5
- Protease Inhibitor tablets (Roche Cat. no. 1873580)
- ZOOM® Focusing Buffers pH 3–7 and pH 7–12
- ZOOM® 2D Protein Solubilizer (recommended), or other sample buffer

  Additional materials for use with ZOOM® 2D Protein Solubilizer:
  - Benzonase from Novagen, Cat. no. 70746-4
  - Tributylphosphine (TBP) from Sigma, Cat. no. T-7567
  - 20% (w/v) SDS
  - **Cold** acetone
  - **Cold** (20°C) 10% (w/v) TCA, 0.07% (v/v) β-mercaptoethanol in acetone (~1 mL/0.3 g tissue) for plant lysate
  - **Cold** (20°C) 0.07% (v/v) β-mercaptoethanol in acetone (~20 mL) for plant lysate

ZOOM® 2D Protein Solubilizer Kit

The ZOOM® 2D Protein Solubilizer Kit (see page 42 for ordering information) includes two sample preparation reagents, Solubilizers 1 and 2, for isoelectric focusing (IEF). We recommend testing both solubilizers with the ZOOM® IEF Fractionator to determine which works best with your proteins.

ZOOM® 2D Protein Solubilizers 1 and 2 are specifically designed to improve solubility of hydrophobic and membrane proteins during sample preparation. They are ready-to-use solutions of proprietary composition (detergents in a chaotrope solution containing urea and thiourea), eliminating the need for time-consuming reagent preparation steps.

ZOOM® Focusing Buffers

ZOOM® Focusing Buffers (pH 3–7 and pH 7–12; see page 42 for ordering information) are proprietary buffer formulations that provide improved focusing and resolution of proteins, especially basic proteins. Each ZOOM® Focusing Buffer is capable of producing a stable pH gradient under electrophoresis conditions. The average molecular weight distribution is 50–300 Daltons.

Protease Inhibitor Cocktail for Preparing Samples

To prepare samples using the ZOOM® 2D Protein Solubilizer Kit, first prepare the following Lysis Buffer. The kit provides two solubilizer solutions, 1 and 2. We recommend testing both solubilizers with your proteins.

1. Prepare a 100X Protease Inhibitor Cocktail by dissolving one Protease Inhibitor Cocktail tablet in 500 µL 1.1X ZOOM® 2D Protein Solubilizer 1 or 2.
2. Mix well and aliquot in smaller volumes. Store aliquots at –20°C.

Continued on next page

Preparing Cell/Tissue Samples

The following procedure uses components of the ZOOM® 2D Protein Solubilizer Kit to prepare cell/tissue samples for IEF fractionation.

1. Prepare the following Lysis Buffer **fresh**, just prior to use. You need 150 µL of Lysis Buffer for each 1 mL of cell/tissue lysate:

100X Protease Inhibitor Cocktail (previous page for recipe)	2 µL
Benzonase (Novagen Cat. no. 70746-4)	100 Units
200 mM Tributylphosphine (Sigma Cat. no. T-7567; use in a chemical hood)	25 µL
99% N,N-Dimethylacrylamide (DMA)	5 µL
40 mM Tris Base	148 µL

   Mix well and store on ice.
2. To 50 mg (wet weight) minced tissue or 50 µL packed *E. coli* (1×10^{10}) cells, add 150 µL chilled Lysis Buffer.
3. Sonicate the sample **on ice** for 5–10 rounds of 15–20 seconds each at ~50% power with cooling samples on ice between sonications. Check pH of the solution. The pH should be 9.0. Adjust pH of the solution with 1 M Tris Base, if needed.
4. Incubate the lysate for 30 minutes at room temperature on a rotary shaker.
5. Add the following to the lysate from Step 4 for reduction and alkylation:

20% (w/v) SDS	200 µL
Deionized Water	to 1 mL

6. Heat at 70°C for 10 minutes. Incubate the lysate on a rotary shaker for 20 minutes at room temperature.
7. Centrifuge at 16,000 × g for 20 minutes at 4°C.
8. Transfer supernatant to a sterile 15 mL tube (rated for 16,000 × g centrifugation). Add 4 mL cold acetone to the supernatant (final acetone concentration is 80%) and incubate at –20°C for 30 minutes for protein precipitation.
9. Centrifuge the lysate at 16,000 × g for 20 minutes at 4°C. Discard supernatant without disturbing the pellet.
10. Wash pellet by resuspending the pellet in ~8 mL cold acetone and gently inverting the tube several times to resuspend the pellet. Centrifuge at 16,000 × g for 20 minutes at 4°C. Discard the supernatant.
11. Allow the pellet to air-dry for 10 minutes at room temperature (avoid overdrying the pellet).
12. Resuspend the pellet in 1 mL ZOOM® 2D Protein Solubilizer 1 or 2. The lysate protein concentration is ~8–10 mg/mL.
13. Proceed to **Diluting Samples for IEF**, page 17, or aliquot supernatant into smaller volumes and store at –80°C.

Continued on next page

Preparing Plant Samples

The following procedure uses components of the ZOOM® 2D Protein Solubilizer Kit to prepare plant samples for IEF fractionation.

1. Prepare the following 950 µL of Lysis Buffer **fresh**, just prior to use.

1.1X ZOOM® 2D Protein Solubilizer 1 or 2	909 µL
1 M Tris Base	3 µL
100X Protease Inhibitor Cocktail	10 µL
2 M DTT	10 µL
Deionized water	18 µL

   Mix well and store on ice.
2. Freeze **plant tissue** in liquid nitrogen in a mortar and grind into a fine powder. To a previously weighed empty microcentrifuge tube, add the ground tissue to determine mass of the tissue.
3. To 100 mg of ground tissue, add ~1 mL of cold 10% (w/v) TCA, 0.07% (v/v) β-mercaptoethanol in acetone, vortex for 1 minute, and then incubate at –20°C for at least 2 hours. Proceed to Step 6, below.
4. If your starting material is **isolated, pelleted chloroplasts**, resuspend chloroplasts in iso-osmotic solution (0.33 M sorbitol). Centrifuge at 10,000 × g for 5 minutes at 4°C to pellet chloroplasts. Discard the supernatant and repeat the wash step once.
5. Determine the mass of pelleted chloroplasts. To 50 mg chloroplasts, add ~1 mL cold 10% (w/v) TCA, 0.07% (v/v) β-mercaptoethanol in acetone. Vortex for 1 minute to resuspend the pellet and incubate at –20°C for at least 2 hours.
6. Centrifuge the precipitated proteins at maximum speed for 20 minutes at 4°C in a microcentrifuge. Discard the supernatant.
7. Resuspend the pellet in 1 mL **cold** acetone containing 0.07% (v/v) β-mercaptoethanol by vortexing. Centrifuge at maximum speed for 20 minutes at 4°C in a microcentrifuge. Discard the supernatant. Repeat washing steps until the pellet is colorless.
8. Discard final supernatant and dry the pellet under vacuum for 2–5 minutes.
9. Resuspend pellet in 950 µL freshly prepared Lysis Buffer from Step 1.
10. Sonicate on ice for 3–6 rounds of 15 seconds each at 20% power. Cool samples on ice between sonications to completely solubilize precipitated proteins. Adjust pH of the solution to 8.4–9.0 with 1 M Tris Base, if needed.
11. Incubate the lysate on a rotary shaker for 10–15 minutes at room temperature.
12. Add 5 µL 99% DMA to the lysate for alkylation. Incubate the lysate on a rotary shaker for 30 minutes at room temperature.
13. Add 10 µL 2 M DTT to quench any excess DMA. Centrifuge at 16,000 × g for 20 minutes at 4°C.
14. Transfer the supernatant to a sterile microcentrifuge tube. Determine the lysate protein concentration and proceed to **Diluting Samples for IEF**, page 17, or aliquot supernatant into smaller volumes and store at –80°C.

Continued on next page

Diluting Samples for IEF

The following procedure uses ZOOM® Focusing Buffers pH 3–7 and pH 7–12 to dilute the prepared sample to 0.6 mg/mL for IEF.

1. Dilute the protein lysate from page 15 or 16 as follows. You need 650 µL of the diluted sample per Sample Chamber in the ZOOM® IEF Fractionator.

   To prepare 1 mL of diluted sample, mix the following.

Component	Amount
Prepared lysate	67–75 µL
1.1X ZOOM® 2D Protein Solubilizer 1 or 2	909 µL
ZOOM® Focusing Buffer, pH 3–7	10 µL
ZOOM® Focusing Buffer pH 7–12	10 µL
2 M DTT	5 µL
Bromophenol blue dye	Trace

   Note: If you are using the Three-Disk Extended pH Range format (see page 19), prepare 650 µL of buffer only without lysate for the pH 9.1-12 sample chamber.

2. Adjust the volume to 1.0 mL with deionized water. The pH of the solution should be ~7.0 (do not adjust pH).
3. The diluted samples are ready to use. Proceed to **Assembling the ZOOM® IEF Fractionator**, next page. You may store the diluted samples at -80°C.

Assembling the ZOOM® IEF Fractionator

Introduction

The ZOOM® IEF Fractionator can be assembled in a number of different configurations, depending on the pH range you want to use.

This section describes the different fractionator configurations and provides step-by-step instructions for assembling the fractionator.

Using the ZOOM® IEF Fractionator for the First Time

The parts of the ZOOM® IEF Fractionator are supplied assembled in the Chamber Assembly Tube.

If you are using the fractionator for the first time, you need to disassemble the fractionator and wash various parts of the fractionator before use as described below:

1. Pull out the Chamber Assembly Tube from the Spill Trough.
2. Unscrew the Cathode End Screw Cap at the end of the Chamber Assembly Tube.
3. Push the protrusion on the Anode End Sealer with your thumb to slide out the Cathode End Sealer and Sample Chambers from the cathode end of the Chamber Assembly Tube.
4. Disassemble the Sample Chambers (remove the caps, O-ring seals, and Spacers) and wash various parts of the fractionator with mild detergent and rinse with deionized water before use.
5. Dry and store the fractionator assembled when not in use.

We recommend having all parts of the ZOOM® IEF Fractionator (Chamber Assembly Tube, Sample Chambers, Sample Chamber Caps, Anode and Cathode End Sealers, Sample Chamber O-ring Seals, Spacers, and Spill Trough) cleaned and on hand before starting.

Prepare cathode and anode buffers as described on next page from the stock solutions included in the ZOOM® IEF Fractionator Combo Kit (recipe is provided on page 41) immediately prior to fractionation. For basic proteins, you will need the ZOOM® Cathode Buffer, pH 9–12.

Materials Needed

You will need the following items. Ordering information is included on page 42.

- ZOOM® Disks (purchased separately or included in the ZOOM® IEF Fractionator Combo Kit)
- Novex® IEF Cathode and Anode Buffers (included in the ZOOM® IEF Fractionator Combo Kit, or a recipe is provided on page 41)
- For basic proteins: ZOOM® Cathode Buffer pH 9–12
- Urea and Thiourea (included in the ZOOM® IEF Fractionator Combo Kit)
- Sodium hydroxide

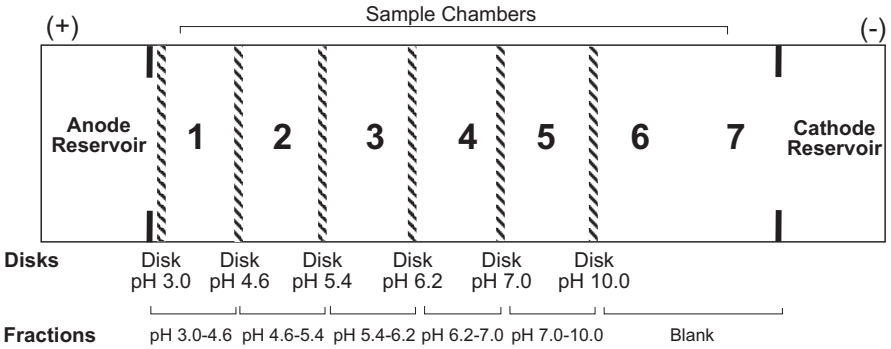
Continued on next page

Fractionator Assembly Formats

The ZOOM® IEF Fractionator can be assembled in a variety of formats, using either ZOOM® Disks or spacers to separate the sample chambers.

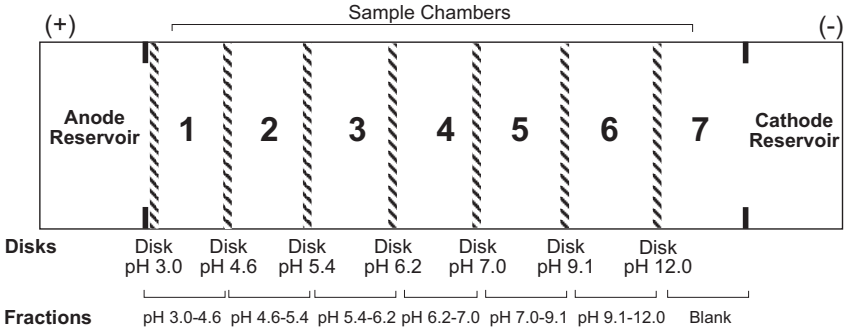
Standard Format

In the example below, six disks are used create five fractions from pH 3.0 to pH 10.0.



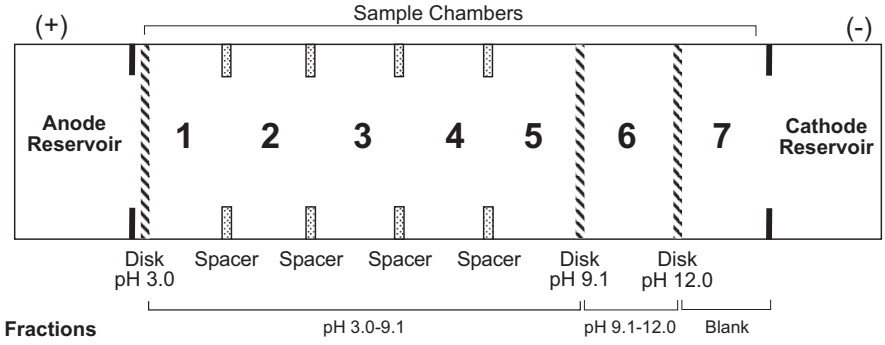
Extended Format

The Extended pH Range format shown below results in six fractions from pH 3.0 to pH 12.0. (**Note:** For this format, you must use the Extended Range Cathode Buffer from page 21.)



Three-Disk Extended Format

The Three-Disk Extended pH Range format shown below results in two fractions, pH 3.0–pH 9.1 and pH 9.1–12.0. (**Note:** For this format, you must use the Extended Range Cathode Buffer from page 21.)



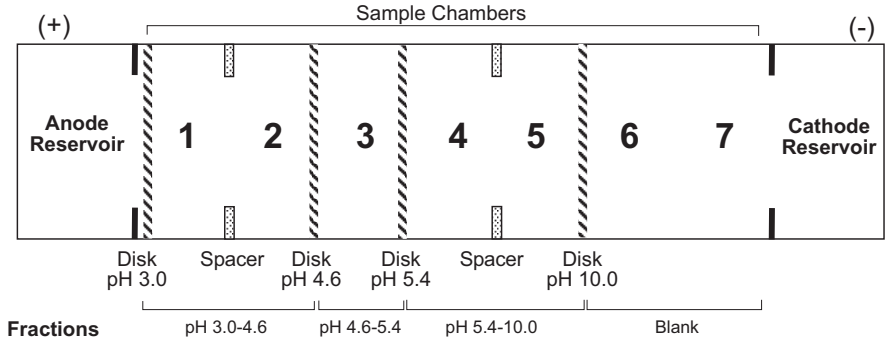
Continued on next page

Fractionator Assembly Formats, Continued

Narrow Range Format

If you are only interested in particular fractions, you can use the disks for the fractions of interest and substitute spacers for the remaining slots between disks. For example, if you are primarily interested in the fraction from pH 4.6 to pH 5.4, you could use the configuration below.

Example of assembly for fractionation between pH 4.6 and 5.4



Examples of Disk/Spacer Combinations

See the table below for fractionator assembly examples using disks and spacers.

Note the following:

- When using disks and spacers in combination, we recommend using no more than a total of six disks/spacers in an assembly. (You can use up to eight disks without spacers in an assembly.)
- When using spacers, we recommend including disks at either end of the assembly (e.g., a pH 3.0 disk at the anode end and either a pH 10.0 or 12.0 disk at the cathode end) to create a pH gradient for efficiently removing the high/low pI proteins away from the fraction of interest.

Fractionation Range	Assembly
pH 4.6–5.4	Anode (+) end, Anode End Sealer, pH 3.0, Spacer, pH 4.6, pH 5.4, Spacer, pH 10.0, Cathode End Sealer, Cathode (-) end
pH 5.4–6.2	Anode (+) end, Anode End Sealer, pH 3.0, Spacer, pH 5.4, pH 6.2, Spacer, pH 10.0, Cathode End Sealer, Cathode (-) end
pH 6.2–7.0	Anode (+) end, Anode End Sealer, pH 3.0, Spacer, pH 6.2, pH 7.0, Spacer, pH 10.0, Cathode End Sealer, Cathode (-) end
pH 3.0–4.6	Anode (+) end, Anode End Sealer, pH 3.0, pH 4.6, Spacer, Spacer, Spacer, pH 10.0, Cathode End Sealer, Cathode (-) end
Extended Range: pH 9.1–12.0	Anode (+) end, Anode End Sealer, pH 3.0, Spacer, Spacer, Spacer, Spacer, pH 9.1, pH 12.0, Cathode End Sealer, Cathode (-) end

Continued on next page

Assembling the ZOOM® IEF Fractionator, Continued

Preparing Cathode and Anode Buffers

Anode Buffer

1. To prepare 20 mL of Anode Buffer, mix the following reagents:

ZOOM® Urea	8.4 g
ZOOM® Thiourea	3.0 g
Novex® IEF Anode Buffer (50X)	3.3 mL
Deionized Water	7.0 mL

2. The pH should be ~2.5–3.0. If needed, adjust to pH 3.0 with Novex® IEF Anode Buffer (50X). Bring the volume up to 20 mL with deionized water.

   Note: The final concentration of Anode Buffer will be 8.25X.

Cathode Buffer—Standard Range (for use with disks up to pH 10.0)

1. To prepare 20 mL of Standard Range Cathode Buffer, mix the following reagents:

ZOOM® Urea	8.4 g
ZOOM® Thiourea	3.0 g
Novex® IEF Cathode Buffer pH 3–10 (10X)	2.0 mL
Deionized Water	to 20 mL

2. The pH should be 10.4. Do not adjust the pH.

Cathode Buffer—Extended Range (for use with disks up to pH 12.0)

For following procedure uses Novex® IEF Cathode Buffer pH 912.

1. To prepare 20 mL of Extended Range Cathode Buffer, mix the following reagents:

ZOOM® Urea	8.4 g
ZOOM® Thiourea	3.0 g
Novex® IEF Cathode Buffer pH 9–12 (10X)	2.0 mL

2. Adjust the pH to 12.0 using sodium hydroxide.
3. Add deionized water to a final volume of 20 mL.

Preparing Sample Chambers

1. Insert the Sample Chamber Caps with O-Ring on the Sample Chambers through the fill-port provided (see Figure A).
2. Place the red Sample Chamber O-Ring Seal on the groove on each Sample Chamber as shown in Figure A. Keep the assembled Sample Chambers (containing the Sample Chamber Cap and O-Ring) on a clean surface.

Figure A

Continued on next page

Assembling the ZOOM® IEF Fractionator, Continued

Assembling the Chamber Assembly Tube

If you are assembling the ZOOM® IEF Fractionator for the first time, you need to disassemble and wash various parts of the fractionator as described on page 18.

Note: You can assemble the tube with various combinations of disks and spacers to cover a pH range of 3.0–10.0, an extended pH range of 3.0–12.0, or narrower pH ranges (e.g., pH 5.4–6.2, pH 9.1–12). See pages 19–20 for format options. All assembly formats should start with a pH 3.0 disk between the Anode End Sealer and the first Sample Chamber, as described in step 3–4 below.

Assemble the ZOOM® IEF Fractionator starting from the anode (+) end as described below:

1. Hold the Chamber Assembly Tube in a vertical position.
2. Insert the Anode End Sealer such that the back end of the Anode End Sealer is facing the bottom of the Chamber Assembly Tube. Slide the Anode End Sealer into the Chamber Assembly Tube until the protrusion of the sealer is flush with the top of the Chamber Assembly Tube (Figure B).
3. Remove ZOOM® Disk pH 3.0 package from the box. Snap one pouch from the perforation and peel off the top cover. Using forceps, remove ZOOM® Disk pH 3.0 from the pouch and blot off excess packaging buffer from the disk by tapping the disk edge on a filter paper. **Avoid touching the active center area of the disks.** **Caution:** The packaging buffer for ZOOM® Disks contains 0.02% sodium azide and residual acrylamide monomer. Wear gloves at all times while handling ZOOM® Disks.
4. Place ZOOM® Disk pH 3.0 on the Anode End Sealer using forceps (Figure C). **Note:** The disk can be placed on the sealer with either side facing down.
5. Place the assembled Sample Chamber (with Sample Chamber Caps and O-ring) on the disk in the Anode End Sealer such that the O-ring is facing the disk (Figure D). Push down on the Sample Chamber until the chamber is flush with the top of the tube.
6. Depending on the assembly format (see page 19), place the next ZOOM® Disk on the chamber (Figure E) using the procedure described in Steps 3–4 above, or place a spacer on the chamber using forceps.
7. Place the second assembled Sample Chamber (with Sample Chamber Caps and O-ring) on the disk such that the O-ring is facing the disk. Push down on the Sample Chamber until it is flush with the top of the Chamber Assembly Tube.

Figure B

Figure C

Figure D

Figure E

Continued on next page

Assembling the ZOOM® IEF Fractionator, Continued

Assembling the Chamber Assembly Tube, continued

8. Repeat Steps 3–7 until all Sample Chambers are serially connected (Figure F) by either disks or spacers. Place disks in order of pH.

   For example: Anode (+) end, pH 3.0, pH 4.6, pH 5.4, pH 6.2, pH 7.0, pH 9.1, pH 12.0, Cathode (-) end.

9. Place an O-ring on the groove of the Cathode End Sealer. Attach the Cathode End Sealer (O-ring on the groove facing down) to the last Sample Chamber (Figure G).

   Note: If the last Sample Chamber is a blank chamber, it does not require a disk or spacer on the cathode (–) end.

10. Screw the Cathode End Screw Cap to the end of the Chamber Assembly Tube (Figure H).

Figure F

Figure G

Figure H

Attaching the Chamber Assembly Tube

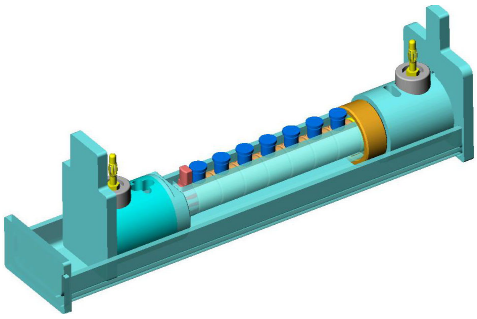
1. Slide the Chamber Assembly Tube into the Spill Trough such that the Cathode End Screw Cap fits into the black O-ringed groove of the cathode reservoir (Figure I).
2. Place the assembled ZOOM® IEF Fractionator on a benchtop in a horizontal position. Proceed to **Loading Samples and Buffers**, next page.

Figure I

Assembled Tube

The figure to the right depicts an assembled ZOOM® IEF Fractionator showing the following parts placed inside the Chamber Assembly Tube:

Anode (+) end, Anode End Sealer, series of seven Sample Chambers with Sample Chamber Caps separated by disks or spacers, Cathode End Sealer, Cathode End Screw Cap, Cathode (–) end.

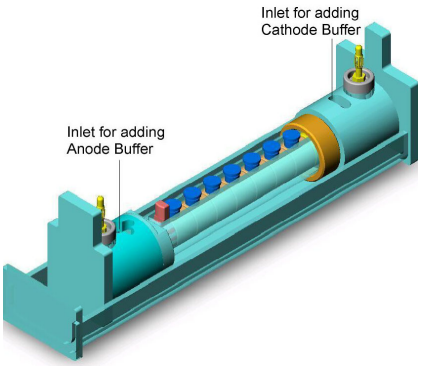


Continued on next page

Assembling the ZOOM® IEF Fractionator, Continued

Loading Anode and Cathode Buffers

After assembling the ZOOM® IEF Fractionator, load the Anode and Cathode Buffers as described below.



1. Load 17.5 mL Anode Buffer (from page 21) into the Anode Reservoir of the Chamber Assembly Tube through the inlet provided (designed as +) using a sterile pipette (see figure right).
2. Load 17.5 mL Cathode Buffer (from page 21) into the Cathode Reservoir through the inlet provided (designed as –) using a sterile pipette (see figure right).

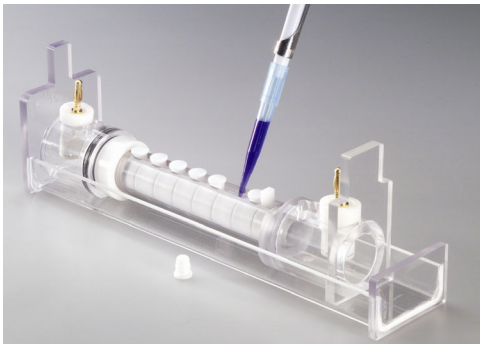
Important: If you are using the pH 12.0 disk, you must use the Extended Range Cathode Buffer (see page 21). If you are using the pH 10.0 disk, use the Standard Range Cathode Buffer (see page 21).

Loading Samples

After you have loaded the Anode and Cathode Buffers, load your samples as follows:

1. Remove the Sample Chamber Cap and add 650 µL of protein sample (from Step 3, page 17) to each Sample Chamber ***except:***
   - Do not add sample to any blank chambers on the cathode end.
   - If you are using the Three-Disk Extended pH Range format (see page 19), add 650 µL of sample buffer only (without lysate) to the pH 9.1–12 sample chamber. To prepare the buffer without lysate, use the preparation procedure on page 17 but do not add lysate.

   Add sample through the fill-port on top of each Sample Chamber (see figure right).

   Note: Avoid trapping any bubbles in the Chamber Assembly Tube or in Sample Chambers. If there are any bubbles, use a gel loading tip to break the bubbles.
2. Insert the Sample Chamber Caps on the Sample Chambers.

⚠ Do not handle the lid if cables are plugged into the power supply.

3. Place the lid on the assembled fractionator.
4. With the power supply turned **off**, connect the electrode cords to the power supply [red to (+) jack, black to (-) jack].

⚠ Make sure that the power is turned off before connecting the ZOOM® IEF Fractionator to the power supply.

5. Turn on the power. See page 25 for **Performing Fractionation**.

Performing Fractionation

Introduction

Once you have assembled the ZOOM® IEF Fractionator and loaded your samples, you are ready to perform fractionation of your samples using solution phase IEF.

You will need a power supply designed for IEF application and capable of operating at low current (see page 10).

To obtain the best results, solution phase IEF is performed by increasing the voltage gradually in steps and maintaining the final voltage for 80 minutes.

Fractionation Conditions

If the power supply has a current and power limiting capability, we recommend setting a current limit at 2 mA and a power limit at 2 W.

1. Perform fractionation with the following conditions:

   100 V for 20 minutes
   200 V for 80 minutes
   600 V for 80 minutes

   Tip: If current is flowing through the system, the bromophenol blue dye from the Sample Chamber migrates towards the anode reservoir resulting in a yellow anode buffer.

   Note: Using 2 mg rat liver lysate prepared as described on page 39 and under the fractionation conditions listed above, the starting current is ~ 0.6 mA, which increases to ~ 1.2 mA at the beginning of the 200 V step, and the ending current is ~ 0.2 mA.

   Samples containing high protein loads, high salt, or high ampholyte concentrations may require longer run times for optimal resolution.

2. While the run is in progress, label sterile 1.5 mL microcentrifuge tubes with the appropriate pH ranges for your fractionated samples.
3. At the end of the run, turn off the power, disconnect cables from the power supply, and proceed immediately to **Disassembling the ZOOM® IEF Fractionator**, next page.

 Do not handle the lid if cables are plugged into the power supply.

Disassembling the ZOOM® IEF Fractionator

Materials Needed

To recover protein adhered to the Sample Chamber walls, you will need the following:

- Pipettor with pipette tips
- 1.1X IEF Denaturant (see page 38 for a recipe)
- 2M DTT

Procedure

Be sure the cables are disconnected from the power supply.

1. Remove the lid from the ZOOM® IEF Fractionator.
2. Remove the Sample Chamber Cap from the Sample Chamber.
3. Using a 1.0 mL pipette tip on a pipettor, retrieve the fractionated sample from each Sample Chamber and transfer the sample to the appropriate 1.5 mL microcentrifuge tube from Step 2, previous page. The volume of sample in each chamber typically ranges from 500 µL to 750 µL.
4. Approximately 20 percent of the protein in each sample will remain adhered to the Sample Chamber walls. To recover this protein, add 293 µL 1.1X IEF Denaturant and 3 µL of 2 M DTT to each chamber. Place the fractionator on a rotary shaker for 10 minutes. Retrieve the wash volume from the chamber and combine with the appropriate fractionated sample from Step 3.
5. Pull out the Chamber Assembly Tube from the Spill Trough. Using a pipette, discard anode and cathode buffers into a waste reservoir.
6. Unscrew the Cathode End Screw Cap at the end of the Chamber Assembly Tube.
7. Push the protrusion on the Anode End Sealer with your thumb to slide out the Cathode End Sealer and Sample Chambers from the cathode end of the Chamber Assembly Tube.
8. Disassemble the Sample Chambers and clean various parts of the device (see next page). Discard the disks.
9. Store the fractionated samples at –80°C or proceed to the downstream application of choice (see **Analyzing Samples from the ZOOM® IEF Fractionator**, page 28).

Important

The ZOOM® Disks are disposable and should be discarded after use. Do not re-use ZOOM® Disks.

Continued on next page

Disassembling the ZOOM® IEF Fractionator, Continued

Cleaning the Device

Wash various parts of the ZOOM® IEF Fractionator with mild detergent and rinse with deionized water. You can autoclave the Sample Chambers and Sample Chamber Caps, if desired. **The Anode and Cathode End Sealers and Cathode End Screw Cap cannot be autoclaved.**

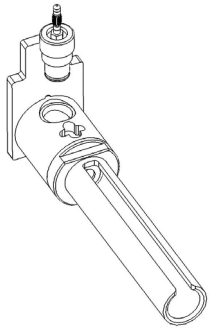
The StainEase® Tray available from Invitrogen (see page 44) is a convenient way to wash and store the small parts (Sample Chambers, O-rings, and Sample Chamber Caps) of the ZOOM® IEF Fractionator.

We recommend storing the Sample Chambers **without** inserting the caps in place.

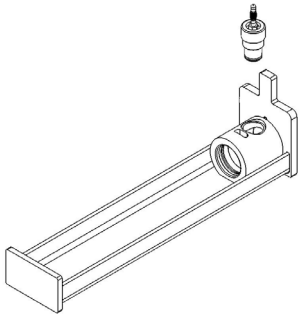
Remove the anode and cathode electrode assembly by unscrewing the electrodes from the buffer reservoirs. Rinse each electrode assembly and buffer reservoir with deionized water after each run to prevent any build up and crystallization of salts (urea and thiourea). After 4–5 uses of the ZOOM® IEF Fractionator, we recommend cleaning the electrode assembly with a mild detergent. After washing, allow the electrode assembly to dry and screw the anode and cathode electrode assembly into the anode and cathode reservoir, respectively.

Do not leave the electrodes in water or cleaning solution overnight.

Anode Electrode Assembly Removed



Cathode Electrode Assembly Removed



Continued on next page

Analyzing Samples from the ZOOM® IEF Fractionator

Introduction

After the sample is fractionated into five fractions based on the pI of proteins, each fraction is suitable for further analysis using established techniques such as one-dimensional electrophoresis, two-dimensional (2D) electrophoresis, or 2D LC/MS. General guidelines for analysis are included in this section.

Concentrating the Sample

We recommend concentrating the fractionated sample prior to one-dimensional electrophoresis and 2D LC/MS analysis. To concentrate the sample:

1. Chill the fractionated sample from step 9, page 26, on ice. Add cold acetone (-20°C) to a final concentration of 80% (v/v) (i.e., add cold acetone at 4X the volume of the fractionated sample).
2. Incubate at -20°C for 30 minutes.
3. Centrifuge the lysate at 16,000 × g for 20 minutes at 4°C.
4. Carefully remove and discard the supernatant, taking care not to disturb the pellet.
5. Allow the pellet to dry for 10 minutes at room temperature. Do not overdry.
6. Dissolve the pellet in the appropriate sample buffer or solvent for your application of choice, and proceed with the application procedure.

One-Dimensional Electrophoresis

You can analyze the fractionated samples by one-dimensional electrophoresis using SDS-PAGE. Prior to analysis, concentrate the sample as described above. We recommend diluting the fractions appropriately with SDS-PAGE sample buffer prior to SDS-PAGE.

A large variety of pre-cast polyacrylamide gels for SDS-PAGE are available from Invitrogen. We recommend using NuPAGE® Novex® Gels to obtain high resolution. For information, visit www.invitrogen.com or contact Technical Support (see page 42).

2D LC/MS Analysis

Prior to 2D LC/MS analysis, concentrate the sample as described above. For more details on sample preparation for 2D LC/MS analysis, contact your core facility or refer to published protocols (Ausubel *et al.*, 1994; Coligan *et al.*, 1998).

Continued on next page

Analyzing Samples from the ZOOM® IEF Fractionator, Continued

Two-Dimensional Electrophoresis

The fractionated sample from the ZOOM® IEF Fractionator is fully compatible with two-dimensional electrophoresis using the ZOOM® IPGRunner™ System. The fractionated sample is in the same buffer required for first dimension IEF using ZOOM® Strips.

We recommend using narrow pH range ZOOM® Strips, which are ~0.1 pH unit wider than the fractionated pools. For example, first dimension IEF of fraction pH 4.6-5.4 from the fractionator is performed using ZOOM® Strip pH 4.5-5.5. For second dimension SDS-PAGE, we recommend using NuPAGE® Novex® ZOOM® Gels.

ZOOM® IPGRunner™ System

The ZOOM® IPGRunner™ System from Invitrogen provides a convenient and quick way to perform IEF of proteins using IPG strips (ZOOM® Strips) for 2D gel electrophoresis of the fractionated samples from the ZOOM® IEF Fractionator. The system utilizes pre-assembled, disposable ZOOM® IPGRunner™ Cassettes for oil-free sample rehydration and performing IEF in one unit. First dimension IEF is complete in less than 4 hours. For more details on the ZOOM® IPGRunner™ System, visit www.invitrogen.com or contact Technical Support (see page 42).

ZOOM® Strips

The ZOOM® Strip is a pre-cast immobilized pH gradient (IPG) gel cast on a plastic backing. ZOOM® Strips are easy to use, yield reproducible pH gradients, and are available in wide and narrow pH ranges (see page 43).

ZOOM® Gels

ZOOM® Gels are 8 × 8 cm, 1.0 mm thick pre-cast polyacrylamide gels cast in a 10 × 10 cm cassette. The ZOOM® Gels are used for 2D analysis of proteins following isoelectric focusing of ZOOM® Strips. ZOOM® Gels contain an IPG well and a molecular weight marker well. The IPG well is designed to accommodate a 7.0 cm IPG strip.

Continued on next page

Performing 2D Electrophoresis with ZOOM® IPGRunner™ System

Recommendations are provided below to perform first dimension IEF of fractionated samples from the ZOOM® IEF Fractionator using the ZOOM® IPGRunner™ System.

For more information on using the ZOOM® IPGRunner™ System, refer to the ZOOM® IPGRunner™ System manual. This manual is available on www.invitrogen.com or from Technical Support (see page 42).

You will need 140 µL of the fractionated sample for each ZOOM® Strip. The recommended ZOOM® Strip for each fraction is listed below.

Fractions	ZOOM® Strips
pH 3.0–4.6	pH 3–10L, pH 3–10NL, pH 4–7
pH 4.6–5.4	pH 4–7, pH 4.5–5.5
pH 5.4–6.2	pH 4–7, pH 5.3–6.3
pH 6.2–7.0	pH 4–7, pH 6.1–7.1
pH 7.0–10.0	pH 6–10, pH 3–10L, pH 3–10NL
pH 9.1–12	pH 9–12

1. To 140 µL of the fractionated sample, add a trace amount of bromophenol blue dye. Use this sample to rehydrate ZOOM® Strips for 1 hour at room temperature as described in the ZOOM® IPGRunner™ System manual.

   Note: We recommend using one cassette for rehydrating and focusing the narrow pH range strips and using a separate cassette for rehydrating and focusing the broad pH range strips. Do not combine broad and narrow pH range strips in one (same) cassette.

2. Apply electrode wicks on the cassette as described in the ZOOM® IPGRunner™ System manual.
3. Assemble the ZOOM® IPGRunner™ Mini-Cell as described in the ZOOM® IPGRunner™ System manual.

Continued on next page

Performing 2D Electrophoresis with ZOOM® IPGRunner™ System, continued

1. Perform IEF using a power supply capable of operating at low current and high voltage as described in the table below. **Note:** The following protocols differ from the standard protocol in the ZOOM® IPGRunner™ System manual. Fractionated samples require longer ramp and run times.

Type of ZOOM® Strip	IEF Protocol
Broad pH range strips: ZOOM® Strip pH 3–10NL ZOOM® Strip pH 3–10L ZOOM® Strip pH 4–7 ZOOM® Strip pH 6–10	175 V for 15 minutes 175–2,000 V ramp for 60 minutes 2,000 V for 60 minutes
Narrow pH range strips: ZOOM® Strip pH 4.5–5.5 ZOOM® Strip pH 5.3–6.3 ZOOM® Strip pH 6.1–7.1	175 V for 15 minutes 175–2,000 V ramp for 60 minutes 2,000 V for 105 minutes
Basic strip: ZOOM® Strip pH 9–12	175 V for 15 minutes 175–2,000 V ramp for 45 minutes 2,000 V for 60 minutes

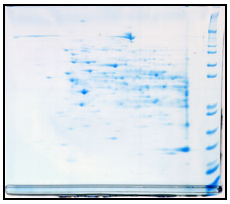
2. After IEF, disassemble the ZOOM® IPGRunner™ Mini-Cell.
3. Proceed to second dimension SDS-PAGE using ZOOM® Gels as described in the ZOOM® IPGRunner™ System manual.

Expected Results of Fractionation

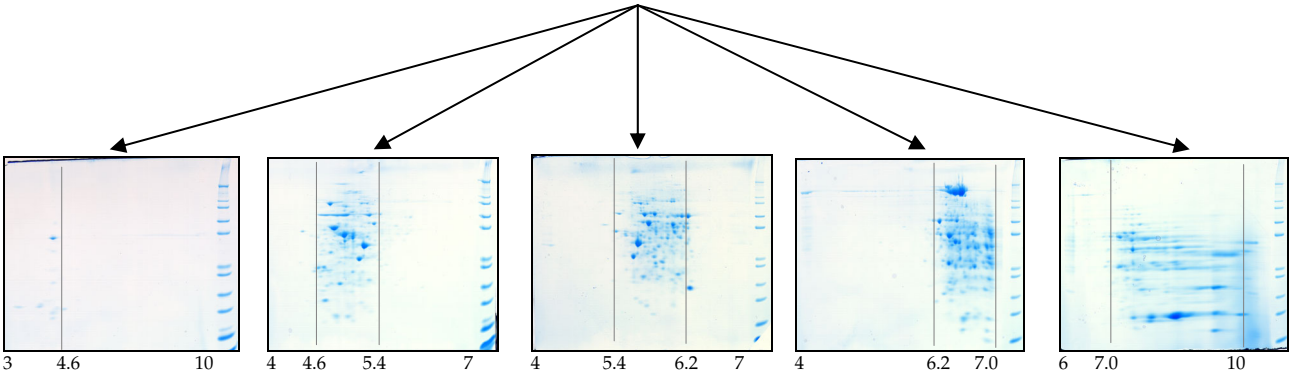
Standard Format

The images below show the results of fractionating rat liver lysate on the ZOOM® IEF Fractionator from pH 3.0 to 10.0 followed by analyzing the fractions by two-dimensional gel electrophoresis. Legend for figures is shown on the next page.

A: Unfractionated Rat Liver Lysate (92 µg)



B: Rat Liver Lysate (2 mg) Fractionated on ZOOM® IEF Fractionator Followed by 2D Electrophoresis



pH 3.0–4.6 fraction on pH 3–10 strip

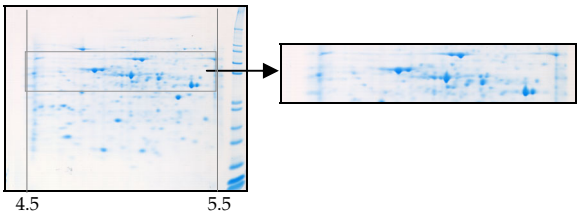
pH 4.6–5.4 fraction on pH 4–7 strip

pH 5.4–6.2 fraction on pH 4–7 strip

pH 6.2–7.0 fraction on pH 4–7 strip

pH 7.0–10.0 fraction on pH 6–10 strip

C: pH 4.6–5.4 Fraction Analyzed on a ZOOM® Strip pH 4.5–5.5



Continued on next page

Expected Results of Fractionation, Continued

Standard Format Figure Legend

Rat liver lysate was prepared, reduced, alkylated, and diluted as described starting on page 38. First dimension IEF was performed on ZOOM® Strips using the ZOOM® IPGRunner™ System. Second dimension SDS-PAGE was performed on NuPAGE® Novex® 4–12% Bis-Tris ZOOM® Gel. Mark12™ Unstained Standard (see page 44) was used as a protein standard for all gels. The gels were stained with SimplyBlue™ SafeStain (see page 44).

Figure A

The diluted rat liver lysate (92 µg) of was analyzed by two-dimension electrophoresis (without fractionation on the ZOOM® IEF Fractionator).

Figure B

The diluted rat liver lysate (2 mg) was subjected to fractionation on ZOOM® IEF Fractionator configured in the Standard Format (see page 19). One hundred and fifty-five microliters of each of the five fractions obtained after fractionation were further analyzed on broad pH range ZOOM® Strips (as indicated under each gel) followed by SDS-PAGE. The proteins obtained from each fraction of the ZOOM® IEF Fractionator are shown between the vertical lines indicated on each gel.

The results demonstrate that the ZOOM® IEF Fractionator efficiently separates a complex lysate into five well-defined fractions. Analysis of each fraction by two-dimensional electrophoresis further enhances the ability to detect low abundance proteins as compared to the unfractionated lysate.

Figure C

An aliquot (155 µL) of the pH 4.6–5.4 fraction of the ZOOM® IEF Fractionator was analyzed on a narrow pH range ZOOM® Strip pH 4.5-5.5. The results indicate the ability to zoom-in on the desired pH range and detect low abundance proteins as compared to the gel shown in figure B.

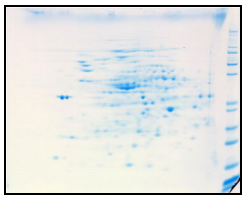
Continued on next page

Expected Results of Fractionation, Continued

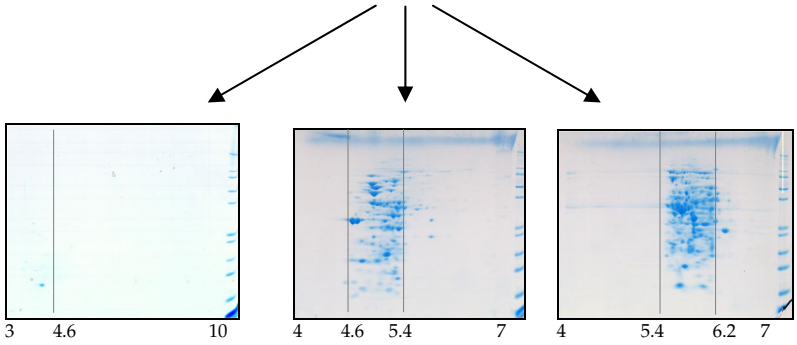
Extended pH Range Format

The images below show the results of fractionating *E. coli* lysate or rat liver lysate on the ZOOM® IEF Fractionator from pH 3.0 to 12.0 followed by analyzing the fractions by two-dimensional gel electrophoresis. Legend for figures is shown on the next page.

A: Unfractionated *E. coli* Lysate (90 µg)



B: *E. coli* Lysate (2 mg) Fractionated on ZOOM® IEF Fractionator Followed by 2D Electrophoresis



pH 3.0–4.6 fraction on pH 3–10 strip

pH 4.6–5.4 fraction on pH 4–7 strip

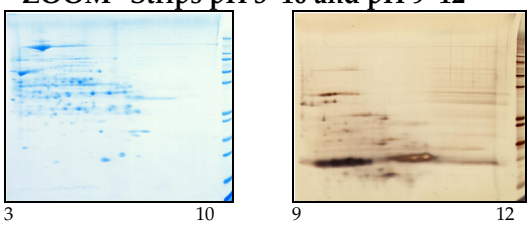
pH 5.4–6.2 fraction on pH 4–7 strip

pH 6.2–7.0 fraction on pH 4–7 strip

pH 7.0–9.1 fraction on pH 6–10 strip

pH 9.1–12.0 fraction on pH 9–12 strip

C: Rat Liver Lysate Separated into pH 3.0–9.1 and pH 9.1–12.0 Fractions and Analyzed on ZOOM® Strips pH 3–10 and pH 9–12



pH 3.0–9.1 fraction on pH 3–10 strip

pH 9.1–12.0 fraction on pH 9–12 strip

Continued on next page

Expected Results of Fractionation, Continued

Extended Format Figure Legend

For Figure A and B, *E. coli* lysate was prepared, reduced, alkylated, acetone precipitated, and diluted as described starting on page 15. First dimension IEF was performed on ZOOM® Strips using the ZOOM® IPGRunner™ System. Second dimension SDS-PAGE was performed on NuPAGE® Novex® 4–12% Bis-Tris ZOOM® Gel. Mark12™ Unstained Standard (see page 44) was used as a protein standard for all gels. The gels were stained with SimplyBlue™ SafeStain or SilverQuest™ Silver Stain (see page 44).

Figure A

The diluted *E. coli* lysate (90 µg) of was analyzed by two-dimensional electrophoresis without fractionation on the ZOOM® IEF Fractionator.

Figure B

The diluted *E. coli* lysate (2 mg) was subjected to fractionation on ZOOM® IEF Fractionator configured in the Extended Format (see page 19). One hundred and fifty-five microliters of each of the five fractions obtained after fractionation were further analyzed on broad pH range ZOOM® Strips (as indicated under each gel) followed by SDS-PAGE. The proteins obtained from each fraction of the ZOOM® IEF Fractionator are shown between the vertical lines indicated on each gel.

The results demonstrate that the ZOOM® IEF Fractionator efficiently separates a complex lysate into six well-defined fractions. Analysis of each fraction by two-dimensional electrophoresis further enhances the ability to detect low abundance proteins as compared to the unfractionated lysate.

Figure C

Diluted rat liver lysate (2 mg) was prepared as described above for *E. coli* lysate and subjected to fractionation on ZOOM® IEF Fractionator configured in the Three-Disk Extended Format (page 19). An aliquot (155 µL) of each fraction from the ZOOM® IEF Fractionator was analyzed on the ZOOM® Strip indicated under each gel. The results indicate the ability to zoom-in on basic proteins in a sample.

Troubleshooting

Introduction

Review the information below to troubleshoot your experiments using the ZOOM® IEF Fractionator.

To troubleshoot 2D electrophoresis using the ZOOM® IPGRunner™ System, refer to the ZOOM® IPGRunner™ System manual available at www.invitrogen.com.

Problem	Cause	Solution
No current for isoelectric focusing	Low current shut-off feature enabled	Check the power supply. Be sure to override the low current shut-off feature as recommended by the manufacturer to enable the power supply to operate at low current.
	Air-bubble in chambers	Avoid trapping any bubbles in the Chamber Assembly Tube or in Sample Chambers. If there are any bubbles, use a gel loading tip to break the bubbles.
Low current or the current read out is 0 mA	It is normal for IEF to proceed at a low current (µA range)	Use proper power supply as recommended on page 10.
High current (initial low current and then increases)	Incorrect buffers used	Use diluted anode and cathode buffers as described on page 21. If you are preparing your own anode and cathode buffers (see page 41 for a recipe), use lysine (free base) and arginine (free base). Do not use lysine HCl and arginine HCl.
	Poor quality reagents used or urea is degraded	Use high-quality, proteomic-grade reagents for sample and buffer preparation. Use freshly prepared urea solutions or stored frozen at –80°C. De-ionize urea solutions on a mixed bed ion exchanger resin using manufacturer's recommendations.
High current (starting current is very high)	Accidentally used wrong buffers in the buffer reservoirs	Use diluted anode and cathode buffers as described on page 21. Add anode buffer to the anode reservoir and cathode buffer to the cathode reservoir as shown on page 24. We recommend using a power supply capable of setting power and current limit to avoid accidental damage to the fractionator due to high currents.
	High salt concentration	Limit the salt concentration in the samples to 10 mM or less.

Continued on next page

Problem	Cause	Solution
The Anode End Sealer is difficult to insert or slips into the Chamber Assembly Tube	Add or remove the friction O-ring	A black friction O-ring is attached to the Anode End Sealer. If the Anode End Sealer is difficult to insert into the Chamber Assembly Tube, remove the black friction O-ring. **Note:** Removal of the friction O-ring may result in sliding of the sealer and Sample Chambers during assembly into the Chamber Assembly Tube. If this results, add the friction O-ring on the Anode End Sealer.
Sample Chamber O-ring Seal does not fit into the Sample Chamber	Sample Chamber is damaged	Inspect the Sample Chamber to check any damage to the Sample Chamber or groove. Use another Sample Chamber included in the Spares Box.
Chamber Assembly Tube difficult to insert or remove from the Spill Trough	Cathode Chamber Seals damaged	Lubricate the Cathode Chamber Seal with silicone by lightly dabbing silicone around the seal with a swab when the Chamber Assembly Tube is difficult to insert or remove from the cathode reservoir. Silicone is typically available in most laboratories. The Chamber Seals (black O-ring seals) on the cathode reservoir may be damaged. Replace with new Chamber Seals included in the Spares Box.
Fractions are not well-defined after fractionation	Leakage between Sample Chambers	Assemble the Sample Chambers in the Chamber Assembly Tube as described on page 22. Improper assembly of the fractionator will not produce proper sealing and result in leaking and contamination of fractions. Be sure to insert the Sample Chamber O-ring Seals on the groove of the Sample Chamber as shown on page 21. Place the ZOOM® Disks on the chamber as shown on page 22.

Appendix

Preparing Samples—Alternative Protocol

Introduction

This section provides an alternative protocol for preparing samples using reagents in the ZOOM® IEF Fractionator Combo Kit. This protocol can be used with the ZOOM® IEF Fractionator assembled in the Standard Format (see page 19) or Narrow Range Formats up to pH 10.0 (page 20). See guidelines for sample preparation starting on page 11.

A recommended sample preparation protocol using ZOOM® 2D Protein Solubilizer and ZOOM® Focusing Buffers is provided starting on page 14.

IEF Denaturant (1.1X)

The following denaturant is used in the protease inhibitor cocktail below and in the sample preparation procedure on the following page.

7.7 M Urea
2.2 M Thiourea
4.4% CHAPS

1. To prepare 40 mL of IEF Denaturant, add the following reagents:

Urea	18.5g
Thiourea	6.7 g
CHAPS	1.8 g

2. Adjust the volume to 40 mL with deionized water. Mix thoroughly. You may warm the solution to 30°C to enhance dissolution.
3. Add 10 mL (~7.2 g) mixed bed ion exchanger resin (Amberlite NB-150; Sigma Cat. no. A-5710) and mix on a rotary shaker until the conductivity is ~ 1 µS/cm (~30-60 minutes).

   Note: Measure the conductivity with a conductivity meter. Refer to the manufacturer for details on calibrating and measuring the conductivity of a sample. If no conductivity meter is available, deionize for 1–2 hours.
4. Allow the resin to settle down at the bottom and collect the supernatant.
5. Filter the supernatant using a 0.2 µm filter. Use polyvinylidene fluoride (PVDF) or nylon filters. Do not use nitrocellulose or cellulose acetate filters.
6. Aliquot the solution into 10 mL aliquots. Store at –80°C.

Continued on next page

Preparing Samples—Alternative Protocol, Continued

Protease Inhibitor Cocktail

Protease inhibitor cocktail tablets are commercially available (Roche Cat. no. 1873580). Dissolve the tablet in a suitable volume of 1.1X IEF Denaturant to obtain 100X protease inhibitor cocktail.

1. If you are preparing your own protease inhibitor cocktail, prepare a 100X solution in 1.1X IEF Denaturant to yield the following final concentration of the proteases:

   AEBSF (4-2-Aminoethyl-benzene sulfonyl-fluoride): 90 mM
   EDTA: 430 mM
   Bestatin: 8.5 mM
   Pepstatin A: 1.4 mM
   E-64: 1.1 mM
   Leupeptin: 1.0 mM
2. Aliquot the solution in small aliquots and store at –80°C.
3. Use the protease inhibitor cocktail at a final concentration of 1X to prepare samples (see below).

Preparing Rat Liver Lysate

A following protocol for preparing rat liver lysate may use be used as a starting point for other lysates. Optimize the procedure based on initial results with your samples. Alternatively, use standard procedures in your laboratory for preparing lysates for IEF.

1. To 50 mg (wet weight) of minced rat liver tissue, add the following in an appropriate tube:

   0.9 mL 1.1X IEF Denaturant (see previous page).

   10 µL 100X protease inhibitor cocktail (see above)

   10 µL 2 M DTT
2. Sonicate the sample on ice for 10 rounds of 20 seconds each, at ~50% power.
3. Add ~10 µL 1 M Tris base (the final pH should be 8.4–8.6).
4. Incubate the lysate for 30 minutes at room temperature.
5. Add 5.2 µL 99% N,N-Dimethylacrylamide (DMA) for alkylation.
6. Incubate the lysate for 30 minutes at room temperature.
7. Add 10 µL 2 M DTT to quench any excess DMA.
8. Incubate the lysate for 5 minutes at room temperature.
9. Centrifuge the lysate at 16,000 × g for 20 minutes at room temperature.
10. After determining the protein concentration of the lysate, proceed to **Diluting Samples for IEF** below, or store the lysate at –80°C in small aliquots.

To determine the protein concentration of the lysate, use the Bradford protein assay method with BSA as the standard. The protein concentration of the lysate should be 8–9 mg/mL.

Continued on next page

Diluting Samples for IEF

After preparing the samples, dilute the reduced and alkylated sample to 0.6 mg/mL for IEF. You will need 650 µL of the diluted sample for each Sample Chamber in the ZOOM® IEF Fractionator.

1. To prepare 1 mL of diluted sample, mix the following:

	0.6 mg/mL
Reduced and alkylated lysate (8–9 mg/mL)	67–75 µL
1.1X IEF Denaturant	0.9 mL
ZOOM® Carrier Ampholytes, pH 3–10	10 µL
2 M DTT	10 µL
Bromophenol blue dye	Trace

2. Adjust the volume to 1.0 mL with deionized water. The pH of the solution should be ~7.0 (do not adjust pH).
3. The diluted samples are ready to use. Proceed to **Assembling the ZOOM® IEF Fractionator**, page 18. If desired, you may store the diluted samples at -80°C.

Additional Recipes

Novex® IEF Cathode Buffer, pH 3–10

The Novex® IEF Cathode Buffer pH 3-10 is available separately (see page 42)

20 mM Lysine (free base)
20 mM Arginine (free base) You can use D, L, or D/L form of arginine
pH 10.1

1. To prepare 100 mL 10X IEF Cathode Buffer pH 3–10, dissolve 2.9 g Lysine (free base) and 3.5 g Arginine (free base) in 100 mL of ultrapure water.
2. Store at 4°C. The buffer is stable for 6 months when stored at 4°C.
3. For fractionation, dilute this buffer as described on page 21.

Novex® IEF Anode Buffer

The Novex® IEF Anode Buffer is available separately (see page 42)

7 mM Phosphoric acid

1. To prepare 100 mL 50X IEF Anode Buffer, mix 2.4 mL 85% phosphoric acid with 97.6 mL ultrapure water.
2. Store at room temperature. The buffer is stable for 6 months when stored at room temperature.
3. For fractionation, dilute this buffer as described on page 21.

Accessory Products

Additional Products for Fractionation

The following products can be used with the ZOOM® IEF Fractionator. For more information or to order, visit or contact Technical Support (see page 45).

Product	Quantity	Catalog no.
ZOOM® Disk pH 3.0	1 pack of 10	ZD10030
ZOOM® Disk pH 4.6	1 pack of 10	ZD10046
ZOOM® Disk pH 5.4	1 pack of 10	ZD10054
ZOOM® Disk pH 6.2	1 pack of 10	ZD10062
ZOOM® Disk pH 7.0	1 pack of 10	ZD10070
ZOOM® Disk pH 9.1	1 pack of 10	ZD10091
ZOOM® Disk pH 10.0	1 pack of 10	ZD10010
ZOOM® Disk pH 12.0	1 pack of 10	ZD10120
ZOOM® Urea	1 kg	ZU10001
ZOOM® Thiourea	1 kg	ZT10002
ZOOM® CHAPS	5 g	ZC10003
Novex® IEF Anode Buffer (50X)	100 mL	LC5300
Novex® IEF Cathode Buffer pH 3–10 (10X)	125 mL	LC5310
ZOOM® Focusing Buffer pH 3–7	10 mL	ZB10037
ZOOM® Focusing Buffer pH 7–12	10 mL	ZB10712
ZOOM® 2D Protein Solubilizer 1	20 mL	ZS10001
ZOOM® 2D Protein Solubilizer 2	20 mL	ZS10002
ZOOM® 2D Protein Solubilizer Kit	1 kit	ZS10003
ZOOM® Cathode Buffer pH 9–12 (10X)	100 mL	ZB10912

Continued on next page

Products for 2D Electrophoresis

The following products can be used for 2D gel electrophoresis. For more information or to order, visit or contact Technical Support (see page 42).

Product	Quantity	Catalog no.
ZOOM® Strip pH 3–10NL	12 strips/pack	ZM0011
ZOOM® Strip pH 3–10L	12 strips/pack	ZM0018
ZOOM® Strip pH 4–7	12 strips/pack	ZM0012
ZOOM® Strip pH 6–10	12 strips/pack	ZM0013
ZOOM® Strip pH 4.5–5.5	12 strips/pack	ZM0014
ZOOM® Strip pH 5.3–6.3	12 strips/pack	ZM0015
ZOOM® Strip pH 6.1–7.1	12 strips/pack	ZM0016
ZOOM® Strip pH 9–12	12 strips/pack	ZM0017
ZOOM® IPGRunner™ Cassettes	10 cassettes	ZM0003
ZOOM® IPGRunner™ Retrofit Kit	1 kit	ZM0004
ZOOM® IPGRunner™ Mini-Cell	1 kit	ZM0001
NuPAGE® Novex® 4–12% Bis-Tris ZOOM® Gel	10 gels/box	NP0330BOX
Novex® 4–20% Tris-Glycine ZOOM® Gel	10 gels/box	EC60261BOX
XCell *SureLock*™ Mini-Cell	1 kit	EI0001
ZOOM® Equilibration Tray	10 trays	ZM0007
NuPAGE® MOPS SDS Running Buffer (20X)	500 mL	NP0001
NuPAGE® MES SDS Running Buffer (20X)	500 mL	NP0002
Novex® Tris-Glycine SDS Running Buffer (10X)	500 mL	LC2675
NuPAGE® LDS Sample Buffer (4X)	250 mL	NP0008
NuPAGE® Sample Reducing Agent	10 mL	NP0009
ZOOM® Carrier Ampholytes pH 4–7	10 mL	ZM0022
ZOOM® Carrier Ampholytes pH 6–9	10 mL	ZM0023
ZOOM® Carrier Ampholytes pH 4–6	10 mL	ZM0025
ZOOM® Carrier Ampholytes pH 5–7	10 mL	ZM0026
ZOOM® Carrier Ampholytes pH 6–8	10 mL	ZM0027
ZOOM® Carrier Ampholytes pH 9–11	10 mL	ZM0024
Novex® Power Supply Adapters	1 set	ZA10001
ZOOM® Dual Power Supply (100–120 VAC 47–60 Hz)	1	ZP10001
ZOOM® Dual Power Supply (220–240 VAC 47-60 Hz)	1	ZP10002

Continued on next page

Protein Markers and Stains

The ordering information for stains and protein markers for 2D gel electrophoresis is provided below.

Product	Quantity	Catalog no.
SilverQuest™ Silver Staining Kit	1 kit	LC6070
SilverXpress® Silver Staining Kit	1 kit	LC6100
Colloidal Blue Staining Kit	1 kit	LC6025
SimplyBlue™ SafeStain	1 L	LC6060
Mark12™ Unstained Standard	1 mL	LC5677
Novex® Sharp Unstained Protein Standard	2 × 250 µL	LC5801
MagicMark™ XP Western Standard	250 µL	LC5600
StainEase® Staining Trays	2/pack	NI2400
SYPRO® Ruby Protein Gel Stain	200 mL	S12001

Technical Support

Web Resources

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our website (www.invitrogen.com).

Corporate Headquarters:
5791 Van Allen Way
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail: tech_support@invitrogen.com

Japanese Headquarters:
LOOP-X Bldg. 6F
3-9-15, Kaigan
Minato-ku, Tokyo 108-0022
Tel: 81 3 5730 6509
Fax: 81 3 5730 6519
E-mail: jpinfo@invitrogen.com

European Headquarters:
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Tech Fax: +44 (0) 141 814 6117
E-mail: eurotech@invitrogen.com

SDS

Safety Data Sheets (SDSs) are available on our website at www.invitrogen.com/sds.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

Purchaser Notification

Limited Use Label License No. 143 ZOOM® IEF Fractionator

This product is the subject of pending patents owned by or licensed exclusively to Invitrogen. The ZOOM® IEF Fractionator was in part developed pursuant to a license from The Wistar Institute of Anatomy and Biology, located in Philadelphia, Pennsylvania. Established in 1892, The Wistar Institute was the first independent medical research facility in the United States. For research use only. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing processes; (2) use of the product or its components to provide a diagnostic or phenotyping service, (3) use of the product to generate information or data for any purpose other than research; (4) use of the product or its components for therapeutic or prophylactic purposes; and (5) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of claims of patents owned by or exclusively licensed to Invitrogen Corporation that are drawn to this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500.

Continued on next page

Limited Warranty

Invitrogen (a part of Life Technologies Corporation) is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, contact our Technical Support Representatives.

All Invitrogen products are warranted to perform according to specifications stated on the certificate of analysis. The Company will replace, free of charge, any product that does not meet those specifications. This warranty limits the Company's liability to only the price of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. The Company reserves the right to select the method(s) used to analyze a product unless the Company agrees to a specified method in writing prior to acceptance of the order.

Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore the Company makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, report it to our Technical Support Representatives.

Life Technologies Corporation shall have no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.

References

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994) *Current Protocols in Molecular Biology*, Greene Publishing Associates and Wiley-Interscience, New York

Bier, M., Egen, N. B., Allgyer, T. T., Twitty, G. E., and Mosher, R. A. (1979) *Peptides: Structure and Biological Functions* (Gross, E., and Meienhofer, J., Eds, Ed.), Pierce Chemical Co., Rockford

Chevallet, M., Santoni, V., Poinas, A., Rouquie, D., Fuchs, A., Kieffer, S., Rossignol, M., Lunardi, J., Garin, J., and Rabilloud, T. (1998) New Zwitterionic Detergents Improve the Analysis of Membrane Proteins by Two- Dimensional Electrophoresis. Electrophoresis *19*, 1901-1909

Coligan, J. E., Dunn, B. M., Ploegh, H. L., Speicher, D. W., and Wingfield, P. T. (1998) *Current Protocols in Protein Science*. Current Protocols (Chanda, V. B., Ed.), John Wiley and Sons, Inc., New York

Faupel, M., Barzaghi, B., Gelfi, C., and Righetti, P. G. (1987) Isoelectric protein purification by orthogonally coupled hydraulic and electric transports in a segmented immobilized pH gradient. J. Biochem. Biophys. Methods *15*, 147-162

Galvani, M., Hamdan, M., Herbert B., and Righetti, P. (2001a) Alkylation Kinetics of Proteins in Preparation of Two-dimensional Maps: A Matrix Assisted Laser Desorption/Ionization-Mass Spectrometry Investigation. Electrophoresis *22*, 2058-2065

Galvani, M., Rovatti, L., Hamdan, M., Herbert B., and Righetti, P. (2001b) Protein Alkylation in the Presence/Absence of Thiourea in Proteome Analysis: A Matrix Assisted Laser Desorption/Ionization-Time of Flight-Mass Spectrometry Investigation. Electrophoresis *22*, 2066-2074

Herbert, B., Galvani, M., Hamdan, M., Olivieri, E., MacCarthy, J., Pedersen, S., and Righetti, P. (2001) Reduction and Alkylation of Proteins in Preparation of Two-dimensional Map Analysis: Why, When, and How? Electrophoresis *22*, 2046-2057

Rabilloud, T. (1998) Use of Thiourea to Increase the Solubility of Membrane Proteins in Two-Dimensional Electrophoresis. Electrophoresis *19*, 758–760

Rabilloud, T. (1999) Solubilization of Proteins in 2-D Electrophoresis: An Outline. Methods Mol. Biol. *112*, 9-19

Rabilloud, T. (2000) Proteome Research: Two Dimensional Gel Electrophoresis and Identification Tools, Springer, Berlin

Righetti, P., Tudor, G., and Gianazza, E. (1982) Effect of 2-mercaptoethanol on pH Gradients in Isoelectric Focusing. J. Biochem. Biophys. Methods *6*, 219-227

Righetti, P. G. (1983) Isoelectric Focusing: Theory, Methodology and Applications, Elsevier, Amsterdam

Continued on next page

References, Continued

Zuo, X., Echan, L., Hembach, P., H.Y., T., Speicher, D. K., Santoli, D., and Speicher, D. (2001) Towards Global Analysis of Mammalian Proteomes Using Sample Prefractionation Prior to Narrow pH Range Two-Dimensional Gels and Using One-Dimensional Gels for Insoluble and Large Proteins. Electrophoresis *9*

Zuo, X., Hembach, P., Echan, L., and Speicher, D. (2002) Enhanced Analysis of Human Breast Cancer Proteomes Using Micro-scale Solution Isoelectrofocusing Combined with High- resolution 1-D and 2-D Gels. Journal of Chromatography *782*, 253-265

Zuo, X., and Speicher, D. (2000) A Method for Global Analysis of Complex Proteomes Using Sample Prefractionation by Solution Isoelectrofocusing Prior to Two-Dimensional Electrophoresis. Anal. Biochem. *284*, 266-278

Zuo, X., and Speicher, D. (2002) Comprehensive Analysis of Complex Proteomes Using Microscale Solution Isoelectrofocusing Prior to Narrow pH Range Two-Dimensional Electrophoresis. Proteomics *2*, 58-68

Notes

Notes

Notes

invitrogen™
by life technologies™

Corporate Headquarters
5791 Van Allen Way
Carlsbad, CA 92008
T: 1 760 603 7200
F: 1 760 602 6500
E: tech_support@invitrogen.com

For country-specific contact information, visit our web site at **www.invitrogen.com**