

USER GUIDE

**novex**<sup>®</sup>  
by *life* technologies<sup>™</sup>

# ZOOM<sup>®</sup> IPGRunner<sup>™</sup> System

For isoelectric focusing of ZOOM<sup>®</sup> Strips

Catalog Numbers ZM0001, ZM0002, ZM0004

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**For Research Use Only. Not intended for any animal or human  
therapeutic or diagnostic use.**

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# Contents

Kit Contents.....	v
Product Specifications.....	vi
Experienced Users Procedure.....	vii
<b>Introduction .....</b>	<b>1</b>
Product Overview .....	1
Description of ZOOM® IPGRunner™ Parts .....	3
Sample Preparation Guidelines .....	6
Experimental Overview.....	12
<b>Methods .....</b>	<b>14</b>
Preparing Samples.....	14
Rehydrating ZOOM® Strips .....	20
Assembling the ZOOM® IPGRunner™ for Focusing.....	23
Performing IEF.....	26
Disassembling the ZOOM® IPGRunner™ .....	28
Performing SDS-PAGE Using ZOOM® Gels .....	29
Expected Results.....	33
Troubleshooting.....	35
<b>Appendix.....</b>	<b>38</b>
Safety Information.....	38
Sicherheits-Anweisungen.....	39
Instructions de securite .....	40
Sample Preparation – Alternative Protocol.....	41
Staining ZOOM® Strips.....	43
Overview of Two-Dimensional Gel Electrophoresis .....	45
First Dimension IEF.....	49
Second Dimension SDS-PAGE .....	51
Detection and Analysis .....	53
Accessory Products .....	55
Technical Support.....	58
Purchaser Notification .....	59
References.....	60



# Kit Contents

## Types of Kits

This manual is supplied with the following kits:

Kit	Catalog no.
ZOOM® IPGRunner™ Mini-Cell	ZM0001
ZOOM® IPGRunner™ Combo Kit	ZM0002
ZOOM® IPGRunner™ Retrofit Kit	ZM0004

## Kit Components

The components included with the different kits are listed below. For more details on each component, see page 3.

Kit	Components
ZOOM® IPGRunner™ Mini-Cell	ZOOM® IPGRunner™ Core (1 each)
	ZOOM® IPGRunner™ Lid (1 each)
	Gel Tension Wedge (1 each)
	Buffer Dam (1 each)
	Mini-Cell Chamber (1 each)
ZOOM® IPGRunner™ Combo Kit (Reagents for sample preparation and second dimension SDS-PAGE are not included)	ZOOM® IPGRunner™ Mini-Cell, (1 each)
	Electrode Wicks (22)
	Sealing Tape (22)
	ZOOM® IPGRunner™ Cassettes (10)
ZOOM® Strip, pH 3–10NL (12)	
ZOOM® IPGRunner™ Retrofit Kit (Compatible with XCell SureLock® Mini-Cell only)	ZOOM® IPGRunner™ Core (1 each)
	ZOOM® IPGRunner™ Lid (1 each)

## Product Use

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

# Product Specifications

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## ZOOM® IPGRunner™ Mini-Cell Specifications

Dimensions:	11 cm × 12 cm × 16 cm
Mini-Cell Chamber Capacity:	650 mL
Material:	Polycarbonate, Acetal, High Density Polyethylene (HDPE)
Electrode Wire:	Platinum (0.010" diameter)
Electrical Limits:	3,500 VDC and 3.5 Watts*
Temperature Limit:	5–40°C

The ZOOM® IPGRunner™ 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 component of the ZOOM® IPGRunner™ System, see page 3.

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## ZOOM® IPGRunner™ Cassette Specifications

Cassette:	10 cm × 10 cm
Thickness:	6.9 mm
Enclosed Channel:	Designed to accommodate 7 cm IPG strips
Cassette Material	Polycarbonate, Polyester, Low Density Polyethylene (LDPE)

For more details on the ZOOM® IPGRunner™ Cassette, see page 3.

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## ZOOM® Strip pH 3–10NL Specifications

Length of the gel:	7.0 cm
Length of the ZOOM® Strip:	7.7 cm
Gel thickness:	0.5 mm
Width of the ZOOM® Strip:	3.3 mm
pH range:	3–10NL (non-linear)
Storage:	Store at –30°C to –10°C
Number of ZOOM® Strips	12/package

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The ZOOM® IPGRunner™ Mini-Cell complies with the European Community Safety requirements. Operation of the ZOOM® IPGRunner™ Mini-Cell is subject to the conditions described in the manual (page 27).

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

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## Maintenance

Wash the ZOOM® IPGRunner™ Mini-Cell with a mild detergent and rinse with deionized water after each use. **Do not wash lid with 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® IPGRunner™ System.

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\*The recommended levels for optimal performance are less than the maximum voltage/wattage given above, see page 26 for details on performing IEF.

# Experienced Users Procedure

## Introduction

This quick reference sheet is provided for experienced users of the ZOOM® IPGRunner™ System. If you are a first time user, follow the detailed methods.

Step	Action
Prepare Sample	Prepare your protein sample at a desired concentration using ZOOM® 2D Protein Solubilizer in a final sample rehydration buffer volume of 140 µL. See preparation guidelines starting on page 6 and recommended procedure starting on page 15 for details.
Rehydrate ZOOM® Strips in the ZOOM® IPGRunner™ Cassette	<ol style="list-style-type: none"> <li>1. Remove the ZOOM® IPGRunner™ Cassette from the box.</li> <li>2. Load 140 µL of the sample rehydration buffer containing an appropriate concentration of protein sample into each Sample Loading Well located at the rounded edge of the ZOOM® IPGRunner™ Cassette.</li> <li>3. Peel a ZOOM® Strip from the card.</li> <li>4. Hold a ZOOM® Strip at the basic end (–) using forceps with the printed side facing <i>down</i> (i.e., gel side up). Using your fingers, gently slide the acidic end (+) of the strip into the sample well at the curved end of the ZOOM® IPGRunner™ Cassette until the acidic end (+) of the strip touches the end of the channel slot.</li> <li>5. Repeat Steps 3–4 for the remaining wells containing sample. Leave unused channels empty.</li> <li>6. Seal all Sample Loading Wells (including unused wells) with the Sealing Tape.</li> <li>7. Incubate the ZOOM® IPGRunner™ Cassette for <b>1 hour</b> at room temperature to rehydrate the ZOOM® Strips. Proceed to <b>Assemble the ZOOM® IPGRunner™ Mini-Cell</b>, immediately following.</li> </ol>
Assemble the ZOOM® IPGRunner™ Mini-Cell	<ol style="list-style-type: none"> <li>1. Remove the Sealing Tape and Sample Loading Devices from the cassette to expose the adhesive.</li> <li>2. Place an Electrode Wick at each end of the ZOOM® IPGRunner™ Cassette over the adhesive, using the black alignment marks to place the wicks.</li> <li>3. Apply 600 µL of deionized water to each Electrode Wick and blot off excess water.</li> <li>4. Slide the cassette into position against the ZOOM® IPGRunner™ Core. The Electrode Wicks will be in contact with the electrodes of the core.</li> <li>5. Repeat Step 4 with a second ZOOM® IPGRunner™ Cassette or use the Buffer Dam in place of a second ZOOM® IPGRunner™ Cassette.</li> <li>6. Slide the sandwich containing the ZOOM® IPGRunner™ Core/ZOOM® IPGRunner™ Cassettes (or Buffer Dam) into the Mini-Cell Chamber of the ZOOM® IPGRunner™ System.</li> <li>7. Insert the Gel Tension Wedge into the Mini-Cell behind the ZOOM® IPGRunner™ Core.</li> <li>8. Pull the Gel Tension lever of the wedge toward the front of the ZOOM® IPGRunner™ Mini-Cell until the lever comes to a firm stop.</li> <li>9. Fill the Outer Chamber of the Mini-Cell with 600 mL of deionized water. <b>Caution: Do not pour any other liquid into the Inner Chamber of the Mini-Cell.</b></li> <li>10. Place the ZOOM® IPGRunner™ Cell Lid on the ZOOM® IPGRunner™ Core. <b>Caution: Do not handle the lid with the electrode cords plugged into the power supply.</b></li> <li>11. With the power supply turned <b>off</b>, connect the electrode cords to the power supply.</li> <li>12. Turn on the power. Proceed to <b>Perform Isoelectric Focusing</b>, page viii.</li> </ol>

*Continued on next page*

## Experienced Users Procedure, continued

Step	Action												
Perform Isoelectric Focusing	<ol style="list-style-type: none"> <li>Perform IEF as follows: (see page 27 for run conditions using step voltage) <table border="1" data-bbox="451 352 1513 516"> <thead> <tr> <th data-bbox="451 352 805 390">Broad Range ZOOM® Strips</th> <th data-bbox="805 352 1159 390">Narrow Range ZOOM® Strips</th> <th data-bbox="1159 352 1513 390">ZOOM® Strips pH 9–12</th> </tr> </thead> <tbody> <tr> <td data-bbox="451 390 805 428">175 V for 15 min</td> <td data-bbox="805 390 1159 428">175 V for 15 min</td> <td data-bbox="1159 390 1513 428">175 V for 15 min</td> </tr> <tr> <td data-bbox="451 428 805 466">175–2000 V ramp for 45 min</td> <td data-bbox="805 428 1159 466">175–2000 V ramp for 45 min</td> <td data-bbox="1159 428 1513 466">175–2000 V ramp for 45 min</td> </tr> <tr> <td data-bbox="451 466 805 504">2000 V for 30 min</td> <td data-bbox="805 466 1159 504">2000 V for 105 min</td> <td data-bbox="1159 466 1513 504">2000 V for 1 hour</td> </tr> </tbody> </table> </li> <li>At the end of the run, turn off power, and disconnect cables from the power supply.</li> </ol>	Broad Range ZOOM® Strips	Narrow Range ZOOM® Strips	ZOOM® Strips pH 9–12	175 V for 15 min	175 V for 15 min	175 V for 15 min	175–2000 V ramp for 45 min	175–2000 V ramp for 45 min	175–2000 V ramp for 45 min	2000 V for 30 min	2000 V for 105 min	2000 V for 1 hour
Broad Range ZOOM® Strips	Narrow Range ZOOM® Strips	ZOOM® Strips pH 9–12											
175 V for 15 min	175 V for 15 min	175 V for 15 min											
175–2000 V ramp for 45 min	175–2000 V ramp for 45 min	175–2000 V ramp for 45 min											
2000 V for 30 min	2000 V for 105 min	2000 V for 1 hour											
Disassemble ZOOM® IPGRunner™	<ol style="list-style-type: none"> <li>Remove the lid and pour off water from the Mini-Cell Chamber.</li> <li>Unlock the Gel Tension Wedge and remove the ZOOM® IPGRunner™ Core/ZOOM® IPGRunner™ Cassette sandwich assembly.</li> <li>Remove the ZOOM® IPGRunner™ Cassette and lay cassettes on a flat surface.</li> <li>Peel off the Film Cover from the ZOOM® IPGRunner™ Cassette. Avoid introducing any liquid on the cassette surface.</li> <li>Remove one ZOOM® Equilibration Tray from the package and remove the adhesive liner. Proceed to <b>Equilibrate ZOOM® Strips</b>, immediately following.</li> </ol>												
Equilibrate ZOOM® Strips	<ol style="list-style-type: none"> <li>Dilute 4X NuPAGE® LDS Sample Buffer to 1X with deionized water.</li> <li>Add 1.0 mL NuPAGE® Sample Reducing Agent (10X) to 9.0 mL 1X NuPAGE® LDS Sample Buffer from Step 1 in a 15-mL conical tube.</li> <li>Place the ZOOM® Equilibration Tray on the cassette and seal tightly.</li> <li>Add 5–15 mL of the buffer with reducing agent from Step 2 through either spout on the tray. Incubate for 15 minutes at room temperature, and then decant the reducing solution.</li> <li>Prepare 125 mM Alkylating Solution by dissolving 232 mg of fresh iodoacetamide in 10 mL of 1X NuPAGE® LDS Sample Buffer (from Step 1, above) in a 15-mL conical tube.</li> <li>Add 5–15 mL of the prepared Alkylating Solution through either spout on the tray.</li> <li>Incubate for 15 minutes at room temperature. Decant the Alkylating Solution and remove the tray from the cassette. Perform SDS-PAGE as described in the following section.</li> </ol>												
Perform SDS/PAGE	<ol style="list-style-type: none"> <li>Prepare 0.5% agarose solution in the appropriate running buffer.</li> <li>Cut the plastic ends of the IPG strip flush with the gel.</li> <li>Slide the ZOOM® Strip into the ZOOM® Gel well and align the ZOOM® Strip properly in the ZOOM® Gel well.</li> <li>Add ~400 µL of 0.5% agarose solution into the ZOOM® Gel well. Allow it to solidify.</li> <li>Assemble the XCell SureLock® Mini-Cell cassette/Buffer Core sandwich.</li> <li>Fill the Lower Buffer Chamber and Upper Buffer Chamber with the appropriate running buffer. Add 0.5 mL of NuPAGE® Antioxidant to the Upper Buffer Chamber.</li> <li>Load molecular weight standards in the marker well.</li> <li>Place the XCell SureLock® Mini-Cell lid on the Buffer Core. Connect electrode cords to the power supply.</li> <li>Perform SDS/PAGE at 200 V for 40–50 minutes for NuPAGE® Novex® Bis-Tris ZOOM® Gel.</li> <li>After electrophoresis, turn off the power, disassemble the cassette/Buffer Core sandwich assembly, and proceed to staining the second dimension gel using a method of choice.</li> </ol>												



# Introduction

## Product Overview

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### Components of the ZOOM® IPGRunner™ System

The ZOOM® IPGRunner™ System provides a convenient and quick way to perform isoelectric focusing (IEF) of proteins using immobilized pH gradient (IPG) strips for two dimensional (2D) gel electrophoresis (see next page for 2D gel electrophoresis). IEF is performed in a vertical mini-gel format using the ZOOM® IPGRunner™ System.

The major components of the ZOOM® IPGRunner™ System are:

- ZOOM® IPGRunner™ Core
- ZOOM® IPGRunner™ Cassette
- ZOOM® IPGRunner™ Lid
- ZOOM® Strips

For more details on each of these components, see page 3.

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### ZOOM® IPGRunner™ Cassette

The ZOOM® IPGRunner™ Cassette is used to perform oil-free rehydration and isoelectric focusing of up to six 7.0 cm IPG strips (ZOOM® Strips) without requiring separate units for rehydration and isoelectric focusing. The ZOOM® IPGRunner™ Cassettes are disposable. See page 3 for more details.

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### ZOOM® IPGRunner™ Core

The ZOOM® IPGRunner™ Core is specifically designed for the ZOOM® IPGRunner™ Mini-Cell and can be used to perform isoelectric focusing of up to 12 IPG strips (ZOOM® Strips) at high voltage. See page 3 for more details.

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### ZOOM® Strips

ZOOM® Strips are immobilized pH gradient gels cast on a plastic backing. The gel on each ZOOM® Strip is 7.0 cm in length. ZOOM® Strips are attached to a tri-fold card for easy removal. See page 5 for more details.

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### Advantages of the ZOOM® IPGRunner™ System

Using the ZOOM® IPGRunner™ System to perform IEF offers the following advantages:

- Oil-free sample rehydration technique due to the enclosed strip and sample loading channels on the ZOOM® IPGRunner™ Cassette (see page 3)
  - Allows rapid rehydration of ZOOM® Strips in 1 hour
  - Permits first dimension IEF in less than 2 hours
  - Ready-to-use, pre-assembled, disposable ZOOM® IPGRunner™ Cassettes for rehydrating and performing IEF in one unit
  - Compatible with existing XCell SureLock® Mini-Cell (ZOOM® IPGRunner™ Retrofit Kit)
  - Unique ZOOM® IPGRunner™ Mini-Cell design for benchtop electrophoresis
  - Eliminates the need to purchase expensive IEF equipment
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## Product Overview, continued

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### 2D Gel Electrophoresis

The steps of 2D gel electrophoresis are described below. For an overview of 2D electrophoresis, see page 45.

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 or IPG strips (ZOOM® Strips) containing a 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 when they reach their isoelectric point. For more details on IEF, see page 49.
2. Second dimension separation of proteins using SDS-PAGE.  
Proteins are separated based on their molecular weight using denaturing polyacrylamide gel electrophoresis (SDS-PAGE). For more details on SDS-PAGE, see page 51.
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. See page 53 for more details on detection methods.
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.

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### Purpose of this Manual

This manual provides the following information:

- Overview of ZOOM® IPGRunner™ System
- Sample preparation guidelines
- Using the ZOOM® IPGRunner™ System for IEF
- Using the XCell SureLock® Mini-Cell for SDS-PAGE
- Expected results
- Troubleshooting

The **Appendix** includes background information and more details on:

- Two-dimensional (2D) gel electrophoresis
- First dimension IEF
- Second dimension SDS-PAGE

**Note:** Detailed protocols for sample preparation and staining the second dimension gel are not included in this manual.

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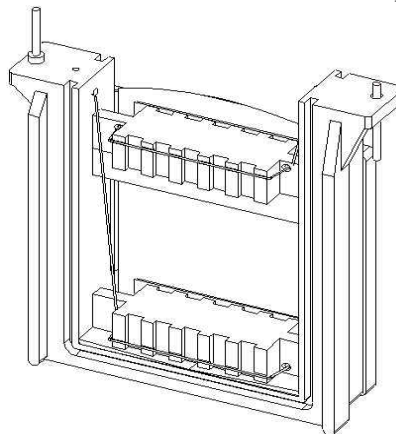
## Description of ZOOM® IPGRunner™ Parts

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### ZOOM® IPGRunner™ Core

The ZOOM® IPGRunner™ Core is used for high voltage isoelectric focusing of up to 12 ZOOM® Strips loaded in two ZOOM® IPGRunner™ Cassettes.

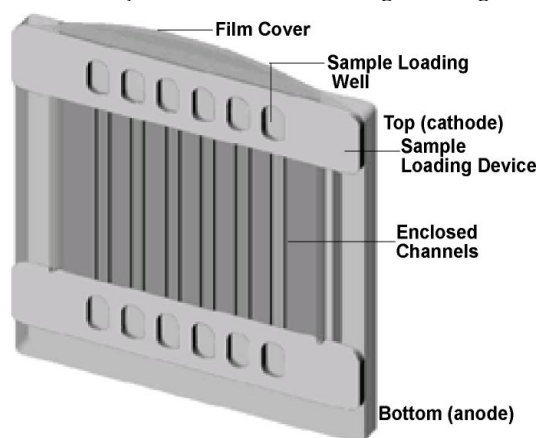
The ZOOM® IPGRunner™ Core is designed to allow firm, even contact between the electrode wire, Electrode Wicks, and the ZOOM® Strips.



### ZOOM® IPGRunner™ Cassette

The ZOOM® IPGRunner™ Cassette (included with ZOOM® IPGRunner™ Combo Kit) is designed to rehydrate ZOOM® Strips and perform isoelectric focusing.

Each cassette contains six Enclosed Channels that receive 7.0 cm ZOOM® Strip's and function as rehydration and running chambers for the strips. Each Enclosed Channel holds rehydration buffer and sample for rehydrating the ZOOM® Strip. There are two Sample Loading Devices attached to the top (cathode) and bottom (anode) end of the ZOOM® IPGRunner™ Cassette. The Sample Loading Wells in the Sample Loading Device are designed for sample application and to prevent any sample overflow into adjacent channels during loading and rehydration.



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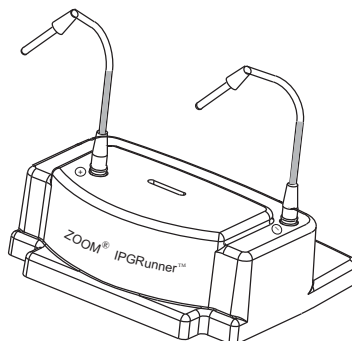
## Description of ZOOM® IPGRunner™ Parts, continued

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### ZOOM® IPGRunner™ Lid

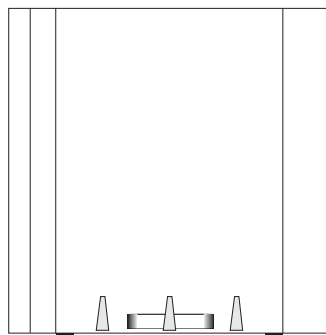
The ZOOM® IPGRunner™ Lid is specifically designed for high voltage electrophoresis. The lid will fit onto the unit in only one orientation to prevent incorrect connection to the electrodes.

**Note:** The ZOOM® IPGRunner™ Lid is not compatible with the Buffer Core used to run SDS-PAGE gels.



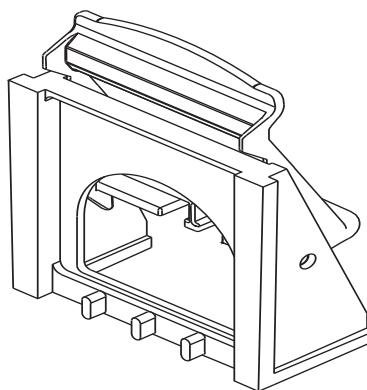
### Mini-Cell Chamber

The Mini-Cell Chamber has a capacity of approximately 600 mL of water and is used as a heat sink during electrofocusing or electrophoresis of proteins.



### Gel Tension Wedge

The positive locking action of the Gel Tension Wedge ensures trouble-free, leak-free electrophoresis of protein gels. Push the lever on the Gel Tension Wedge forward into a locked position to generate an even horizontal force which seals the ZOOM® IPGRunner™ Cassette / ZOOM® IPGRunner™ assembly firmly into position in the Mini-Cell Chamber (see page 25 for an illustration of the locked position).



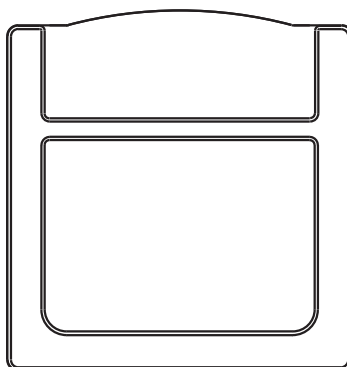
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## Description of ZOOM<sup>®</sup> IPGRunner<sup>™</sup> Parts, continued

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### Buffer Dam

Use the Buffer Dam when you need to run only one ZOOM<sup>®</sup> IPGRunner<sup>™</sup> Cassette.

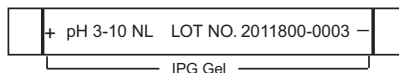


### ZOOM<sup>®</sup> Strips

ZOOM<sup>®</sup> Strips are immobilized pH gradient gels cast on a plastic backing. The gel on each ZOOM<sup>®</sup> Strip is 7.0 cm long and 3.3 mm wide. The unique formulation of ZOOM<sup>®</sup> Strips allows rapid rehydration of strips in 1 hour unlike traditional overnight rehydration.

The ZOOM<sup>®</sup> Strip pH 3–10NL, included with ZOOM<sup>®</sup> IPGRunner<sup>™</sup> Combo Kit, contains a non-linear (NL) pH gradient. The pH gradient is expanded from pH 4–7, forming a sigmoidal pH gradient. ZOOM<sup>®</sup> Strips with different pH gradients are available separately (see page 55).

The plastic backing side of each strip indicates the pH gradient, lot number, and acidic (+) and basic (–) ends of the strip. The last four digits of the lot number on each ZOOM<sup>®</sup> Strip is a unique serial number, allowing you to track multiple samples.



# Sample Preparation Guidelines

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## Introduction

Proper sample preparation is key to the success of a 2D experiment. Various factors affect the design of a sample preparation protocol. General guidelines for sample preparation are provided in this section.

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

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## Important

Due to the large variety of proteins present in different cells and tissues, it is not possible to have a single 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. Optimization of sample preparation can easily and rapidly be achieved using the ZOOM® IPGRunner™ System.

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

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## Objectives of Sample Preparation

The major objectives of sample preparation are to:

- Completely solubilize proteins
  - Denature proteins
  - Reduce disulfide bonds in proteins
  - Prevent protein modification
  - Remove interfering components
  - Maintain proteins in solution during rehydration and IEF
- 

## 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 proceeding for sample preparation. Knowledge about your sample helps in determining the method of extraction, choice of sample rehydration buffer, and type of interfering components present in the sample.
  - The purpose of the 2D experiment is helpful in deciding the protein load and need for sample prefractionation. If the goal is to study the complete protein profile of the sample, low to medium protein load of the total lysate is recommended. If the goal is to study the low abundance proteins present in the sample, a high protein load of a prefractionated or enriched sample is recommended.
  - Contaminants such as salts, conductive ions, nucleic acids, and insoluble materials will affect 2D electrophoresis. Removal of these contaminants using dialysis, centrifugation, or precipitation improves the resolution on the 2D gel. **Note:** Performing multiple steps to improve the sample quality may result in loss of some proteins.
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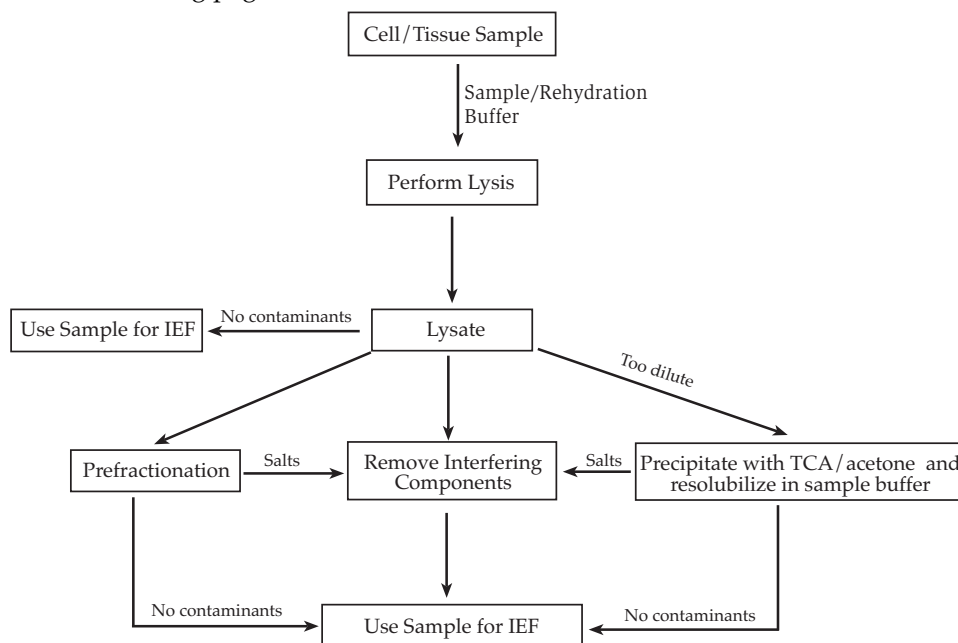
## Sample Preparation Guidelines, continued

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### General Strategy and Guidelines

#### Strategy

The general strategy for sample preparation is outlined below. For more details, see the following pages.



#### Guidelines

General guidelines for sample preparation are listed below:

- Use a method of choice to prepare your protein sample at a suitable concentration using an appropriate sample rehydration buffer
- Prepare samples fresh or use samples stored at  $-85^{\circ}\text{C}$  to  $-68^{\circ}\text{C}$
- Avoid freezing and thawing of samples or heating samples above  $30^{\circ}\text{C}$  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 interfering components from the sample as described on page 10
- Maintain the salt concentration of the sample at  $<10$  mM
- If possible, try to prepare samples in sample rehydration buffer by optimizing components or concentrations of the sample rehydration buffer

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## Sample Preparation Guidelines, continued

### Sample Rehydration Buffer

The sample rehydration buffer, also as known as the sample buffer, is used to denature and solubilize protein samples, and rehydrate the IPG strips. The first step in sample preparation is selecting and/or preparing a suitable sample rehydration buffer.

Due to the large variety of proteins, there is no universal sample rehydration buffer. The starting material and goal of the experiment are two factors to consider when selecting a buffer.

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

The table below lists the major components of the sample rehydration buffer, their function, and their recommended concentration. Note that 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
<p><b>Urea</b> Urea solutions are prepared fresh or stored frozen at <math>-30^{\circ}\text{C}</math> to <math>-10^{\circ}\text{C}</math>. De-ionize urea solutions on a mixed-bed ion exchange resin using manufacturer's recommendations. Thiourea is used to increase solubility of some proteins (Rabilloud, 1998)</p>	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
<p><b>Detergent</b> 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.</p>	Solubilizes the proteins and helps to maintain the proteins in solution during rehydration and IEF	Total detergent concentration range is 0.5–4%
<p><b>Reducing Agent</b> Use DTT or DTE (dithioerythritol). <math>\beta</math>-mercaptoethanol is not recommended for reduction (Righetti et al., 1982).</p>	Cleaves the disulfide bonds in the proteins	DTT or DTE at 20 mM to 100 mM
<p><b>Ampholytes</b> Use the appropriate carrier ampholytes based on the pH range of the IPG strip. May use ampholytes pH 3–10 for all pH ranges of IPG strips.</p>	Helps in solubilizing proteins and aids in maintaining the pH gradient	0.2–2% <b>Note:</b> Higher ampholyte concentration requires longer focusing times.

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## Sample Preparation Guidelines, continued

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### Sample Rehydration Buffer, continued

ZOOM® 2D Protein Solubilizers are specifically designed to improve the solubility of hydrophobic and membrane proteins during sample preparation. The ZOOM® 2D Protein Solubilizer Kit includes two formulations (Solubilizer 1 and 2) that may be used to prepare a sample buffer for a wide variety of proteins. We recommend trying them with your samples (see an example procedure starting on page 14). A recipe for denaturing sample rehydration buffer is also provided on page 41.

**Note:** To obtain high resolution, IEF is usually performed under denaturing and reducing conditions. Use a denaturing sample rehydration buffer for sample preparation.

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### Preparing Extracts

Preparing cell/tissue extracts is an important step in sample preparation. You need to use a procedure that will release all proteins into the sample rehydration buffer with minimal proteolysis to maintain sample integrity. The method of cell/tissue extraction is dependent on the starting material. Use an appropriate sample rehydration buffer (see page 8).

Protease inhibitors may be added to the buffer to prevent proteolysis. You may use individual protease inhibitors for each class of protease or use a mixture (cocktail) of protease inhibitors that is effective against all proteases. Protease inhibitor cocktails are commercially available. Refer to manufacturer's recommendation for the final concentration of the protease inhibitor.

General guidelines are provided below to prepare extracts from various sources, and example procedures are provided starting on page 14. You may use these guidelines and procedure as a starting point and then optimize your procedure based on the initial results.

Also, we recommend that you review published literature for preparing extracts (Bollag et al., 1996; Deutscher, 1990; Rabilloud, 1999).

- **Bacteria, Yeast, Insect, and Mammalian Cells**

Grow cells using standard conditions. After cells have reached the desired density, harvest cells by centrifugation. Wash cells with low salt buffer to remove any residual media components. Resuspend the cell pellet in sample rehydration buffer (usually containing denaturant, detergent, and reducing agent) and perform lysis by sonication (bacteria) or glass beads (yeast). This lysate can be used for IEF or stored at  $-85^{\circ}\text{C}$  to  $-68^{\circ}\text{C}$  in aliquots. References for preparing extracts from various sources include: bacteria (Cull & McHenry, 1990), yeast (Jazwinski, 1990), plants (Geigenheimer, 1990), and membrane proteins (Molloy, 2000).

- **Animal and Plant Tissues**

For soft tissue, cut the tissue into small pieces and homogenize the tissue with a homogenizer/tissue grinder using a sample buffer containing denaturant, detergent, and reducing agent. For hard tissue, freeze the tissue in liquid nitrogen and grind the tissue to a powder. Resuspend the powder in sample rehydration buffer containing denaturant, detergent, and reducing agent. Centrifuge the lysate at high speed to remove any insoluble particles.

Plant tissues contain phenols that may modify proteins by oxidation. To remove plant phenols from the sample, perform a precipitation step (Flengsrud & Kobro, 1989).

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*Continued on next page*

## Sample Preparation Guidelines, continued

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### Preparing Extracts, continued

After preparing lysates, you may need to remove interfering components such as salts from the sample. See below for details.

To further fractionate the lysate, see page 11, **Prefractionating Extracts**.

---

### Removing Interfering Components

Many components present in the sample after sample preparation may interfere with 2D electrophoresis, causing streaking, longer run times, poor focusing, high conductivity, and protein modification. To obtain good resolution, it is important to remove these interfering components prior to IEF.

The most common interfering components and the recommended methods to remove these components are described below.

- **Charged Molecules**

These include salts (e.g., NaCl, KCl), buffer components (e.g., Tris, PBS), and small, charged molecules (e.g., nucleotides). **Salt is the major cause of poor focusing in IEF.** The presence of salt and charged molecules increases focusing time, as proteins will migrate to their pI only after the ions have moved out of the strips. Burning of strips (arcing) can occur when the salt concentration is very high.

The charged molecules are easily removed by dialysis or gel filtration.

- **SDS**

Proteins form complexes with SDS. The resultant SDS-protein complexes are highly negatively charged and move towards the positive electrode. Performing acetone precipitation helps to reduce the SDS concentration in the samples. If low concentrations of SDS are used for sample preparation, dilute the SDS to a final concentration of 0.25–0.5% in the sample rehydration buffer or maintain the ratio of non-ionic/zwitterionic detergent (NP-40 recommended) to SDS between 5:1–8:1 in the sample to minimize effects of SDS on IEF.

- **DNA**

DNA is negatively charged and binds to proteins resulting in artifacts in protein migration during IEF. Because DNA is high-molecular weight, it will decrease movement of proteins into the gel during rehydration by clogging the gel pores. If detection of 2D gel is performed using silver staining, DNA may also stain contributing to background staining.

DNA is removed from the sample using DNase treatment or sonication.

Remember to account for DNase spot on the 2D gel as DNase is a protein and may appear as a distinct spot on the gel.

- **Particulate material**

Remove particulate materials from the sample by high-speed centrifugation.

- **Serum proteins**

Serum proteins such as albumin and immunoglobulin (IgG) constitute ~75% of total serum proteins. Removal of these abundant proteins allows detection of low abundance proteins present in serum. Albumin and IgG are removed from serum using affinity chromatography (Lollo et al., 1999) or Cibacron Blue Dye chromatography.

For detailed protocols on dialysis, gel filtration, affinity chromatography, and precipitation, see published literature (Ausubel et al., 1994; Coligan et al., 1998).

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*Continued on next page*

## Sample Preparation Guidelines, continued

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### **Prefractionating Extracts**

Sample prefractionation methods prior to 2D electrophoresis allow fractionation of complex mixture of proteins into smaller defined fractions. Sample prefractionation improves the detection of low abundance proteins by lowering complexity of the sample, enriching for the protein of interest, and allowing higher protein loads.

The different methods of prefractionation are:

- **Solution phase isoelectric focusing**

This method reproducibly fractionates cell lysates into well-resolved fractions based on the pI using solution phase isoelectric focusing (IEF) with the ZOOM® IEF Fractionator (page 16). The resolved fractions are loaded on narrow pH range IPG strips followed by 2D gel electrophoresis to obtain detailed protein profiles of various proteomes (Zuo & Speicher, 2000).

- **Subcellular fractionation**

Lysates are fractionated into different subcellular fractions (e.g., nuclei, lysosomes, microsomes, and membranes) using centrifugation (Huber et al., 1996).

- **Conventional chromatographic techniques**

Chromatographic techniques such as ion-exchange chromatography, gel-filtration, or affinity chromatography are used. See Deutscher, 1990 for more details.

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### **Protein Estimation**

Use an accurate and sensitive protein estimation method. Note that many components of the sample rehydration buffer (urea, detergent, DTT, ampholytes) may interfere with protein estimation methods. Choose a protein estimation method that is insensitive to these components. We recommend using the Qubit® Protein Assay Kit (for ordering information see page 56) for easy and sensitive fluorescence-based quantitation of proteins.

Accurate protein estimation is essential for calculating protein load and performing subsequent detection. Any error in protein estimation may result in no spots or missing spots on the second dimension SDS gel.




We do not recommend estimating protein concentration using UV absorption.

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# Experimental Overview

## Flow Chart

The flow chart below describes the steps for performing 2D gel electrophoresis using the ZOOM® IPGRunner™ System.

Step	Procedure	Time	Page
1	Prepare protein samples in a final volume of 140 µL.	Variable	14
2	Apply rehydration buffer containing the sample into ZOOM® IPGRunner™ Cassettes and insert ZOOM® Strips into ZOOM® IPGRunner™ Cassettes. Seal loading wells. 	10 minutes	21
3	Rehydrate strips.	1 hour	21
4	Remove wells, apply wicks, and assemble the ZOOM® IPGRunner™ Mini-Cell.	20 minutes	23–24
5	Perform IEF. 	90 minutes	27
6	Equilibrate ZOOM® strips for 2D SDS-PAGE, insert strips into a ZOOM® Gel, and assemble XCell SureLock® Mini-Cell.	35 minutes	30–32
7	Perform SDS-PAGE. 	40 minutes	32
8	Stain 2D gel using the SilverQuest™ Silver Staining Kit or SimplyBlue™ SafeStain.	90 or 45 minutes	53

*Continued on next page*

## Experimental Overview, continued

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### Materials Needed

You will need the following items. Ordering information is provided starting on page 55.

- Power supply (see below for specifications)
- Forceps

### Sample Preparation

- ZOOM® 2D Protein Solubilizer (*optional*: Sample Rehydration Buffer; see page 41 for a recipe if you are preparing your own buffer)

### Optional: Sample Fractionation

- ZOOM® IEF Fractionator and associated reagents

### First Dimension IEF

If you have ordered cat. nos. ZM0001 or ZM0004, you will also need:

- ZOOM® IPGRunner™ Cassettes
- ZOOM® Strips

The ZOOM® IPGRunner™ Cassette is also compatible with 7.0 cm IPG strips available from Bio-Rad and GE Healthcare.

### Second Dimension SDS-PAGE

- 4X NuPAGE® LDS Sample Buffer
- NuPAGE® Sample Reducing Agent
- ZOOM® Gel
- ZOOM® Equilibration Tray
- 0.5% agarose solution in running buffer
- XCell SureLock® Mini-Cell
- Protein molecular weight marker (Mark12™ Unstained Standard)

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### Power Supply Specifications

The ZOOM® IPGRunner™ System is used with 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.
- Be programmable, with a 4-protocol minimum.
- Be able to operate at low current (<1 mA) as IEF is performed at very low current (see the following **Note**).

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### Note

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 (page 55). Contact the manufacturer for information about your power supply.

The electrical leads of the ZOOM® IPGRunner™ Lid are recessed and may not fit into some power supply units. To allow connection of the ZOOM® IPGRunner™ power leads with certain power supplies, use Novex® Power Supply Adapters available separately (page 55) or contact Technical Support (see page 58).

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# Methods

## Preparing Samples

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### Introduction

Proper sample preparation is key to the success of a 2D experiment. Various factors affect the design of a sample preparation protocol.

A procedure for sample preparation using the ZOOM® 2D Protein Solubilizer Kit is provided in this section. You may also prepare samples using your method of choice. See page 6 for guidelines, and page 38 for an alternative sample preparation protocol.

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### Important

Review the **Sample Preparation Guidelines** starting on page 6 prior to sample preparation. Follow recommendations on the next page to obtain the best results.

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### Note

The ZOOM® IPGRunner™ System is designed to perform IEF under denaturing and reducing conditions. We do not recommend using this system under non-reducing conditions.

If you need to perform IEF without DTT (non-reducing conditions) using the ZOOM® IPGRunner™ System, you may need to optimize the run parameters based on your initial results.

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### Choosing a Sample Preparation Method

This manual provides information about the following sample preparation methods:

Method	Page
ZOOM® 2D Protein Solubilizer Kit (separate procedures are provided for cell/tissue samples and plant samples)	16
Sample Fractionation (ZOOM® IEF Fractionator)	16
Sample Rehydration Buffer (buffer recipe and protocol are provided)	38

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### Materials Needed

- Protein sample for IEF
  - ZOOM® 2D Protein Solubilizer, or your own sample rehydration buffer (see page 41 for a recipe if you are preparing your own buffer)  
Additional materials for use with ZOOM® 2D Protein Solubilizer:
    - Protease Inhibitor tablets (Roche Cat. no. 1873580)
    - 1 M Tris Base
    - 2 M DTT
    - N,N-Dimethylacrylamide (DMA) (Aldrich, Cat. no. 27413-5)
    - **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
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*Continued on next page*

## Preparing Samples, continued

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To obtain the best results, follow the recommendations listed below:

- Use fresh sample rehydration buffer or use buffer that is stored frozen at  $-20^{\circ}\text{C}$  in aliquots
  - Prepare samples just prior to IEF or store samples frozen at  $-85^{\circ}\text{C}$  to  $-68^{\circ}\text{C}$  in smaller aliquots to maintain protein representation
  - Avoid repeated freezing and thawing of protein samples
  - **Do not** heat protein samples containing urea as heating in the presence of urea results in protein carbamylation (Righetti, 1983)
  - Use de-ionized urea (see page 8).
  - Make sure the protein sample remains in solution at room temperature
  - Use high-quality reagents and ultrapure water for preparing your samples and rehydration buffer
  - Avoid diluting the sample rehydration buffer with sample because dilution may cause protein precipitation (most proteins require 8M urea for solubility)
  - Maintain the salt concentration in samples to  $<10$  mM as high-salt samples require longer run times due to increased conductivity
- 

### Amount of Protein

We recommend loading 5–15  $\mu\text{g}$  (for silver staining) or 20–50  $\mu\text{g}$  (for Coomassie staining) of total protein per ZOOM<sup>®</sup> Strip. Higher amounts of sample may be loaded on narrow pH range ZOOM<sup>®</sup> Strips. For the ZOOM<sup>®</sup> Strip pH 9–12, we recommend loading 50–100  $\mu\text{g}$  (silver staining) or 100–200  $\mu\text{g}$  (Coomassie staining) of total protein per strip.

If you are using a fractionated or partially purified protein sample, you may load up to 400  $\mu\text{g}$  of total protein per strip.

You may increase the protein load after optimizing sample preparation protocol and focusing parameters.

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### Volume of Diluted Sample

You will need 140  $\mu\text{L}$  of sample diluted in sample rehydration buffer to rehydrate each ZOOM<sup>®</sup> Strip for the standard rehydration time of 1 hour. If you want to extend the rehydration time to overnight, you will need 155  $\mu\text{L}$  of diluted sample.

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*Continued on next page*

## Preparing Samples, continued

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### **ZOOM® 2D Protein Solubilizer Kit**

The ZOOM® 2D Protein Solubilizer Kit includes two sample preparation reagents, Solubilizers 1 and 2, for isoelectric focusing (IEF) and two-dimensional (2D) electrophoresis. We recommend testing both solubilizers with the ZOOM® IPGRunner™ System 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. Samples prepared with ZOOM® 2D Protein Solubilizers and analyzed by 2D electrophoresis show increased spot resolution, less streaking, and more intense spots.

Procedures for using the ZOOM® 2D Protein Solubilizer Kit with cell/tissue samples and plant samples are provided on pages 17–19.

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### **Sample Fractionation**

The ZOOM® IEF Fractionator (see page 56 for ordering information) provides a simple and convenient method to reproducibly fractionate cell and tissue lysates into well-resolved fractions on the basis of isoelectric point using solution-phase IEF.

You can perform fractionation using solution-phase IEF in small volumes combined with 2D gel electrophoresis on narrow pH range strips to generate detailed protein profiles of various proteomes. 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
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## Preparing Samples, continued

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### Lysis Buffer for Preparing Samples

To prepare samples using the ZOOM<sup>®</sup> 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.

**Note:** A Lysis Buffer volume of 950  $\mu\text{L}$  is sufficient for 50 mg (wet weight) minced tissue, 50  $\mu\text{L}$  packed *E. coli* ( $1 \times 10^{10}$ ) cells, or 100 mg ground plant tissue.

1. Prepare a 100X Protease Inhibitor Cocktail by dissolving one Protease Inhibitor Cocktail tablet (Roche Cat. no. 1873580) in 500  $\mu\text{L}$  1.1X ZOOM<sup>®</sup> 2D Protein Solubilizer 1 or 2.
2. Mix well and aliquot in smaller volumes. Store aliquots at  $-30^{\circ}\text{C}$  to  $-10^{\circ}\text{C}$ .
3. Prepare 950  $\mu\text{L}$  of Lysis Buffer using the following components. Prepare this buffer **fresh**, just prior to use.

1.1X ZOOM <sup>®</sup> 2D Protein Solubilizer 1 or 2	909 $\mu\text{L}$
1 M Tris Base	3 $\mu\text{L}$
100X Protease Inhibitor Cocktail	10 $\mu\text{L}$
2 M DTT	10 $\mu\text{L}$
Deionized water	18 $\mu\text{L}$
Total volume	950 $\mu\text{L}$

4. Mix well and store on ice until use.
- 

### Preparing Cell/Tissue Samples

The following procedure uses components of the ZOOM<sup>®</sup> 2D Protein Solubilizer Kit to prepare cell/tissue samples for 2D analysis.

1. Add the 950  $\mu\text{L}$  chilled Lysis Buffer from Step 4, above, to 50 mg (wet weight) minced tissue or 50  $\mu\text{L}$  packed *E. coli* ( $1 \times 10^{10}$ ) cells.
2. Sonicate sample **on ice** for 5–10 rounds of 15 seconds each at ~50% power. Cool the sample on ice between sonications.
3. Check the pH of the solution. The pH should be 8.4–9.0. Adjust the pH with 1 M Tris Base, if needed.
4. Incubate lysate on a rotary shaker for 10–15 minutes at room temperature.
5. Add 5  $\mu\text{L}$  99% N,N-Dimethylacrylamide (DMA) to the lysate for alkylation. Incubate the lysate on a rotary shaker for 30 minutes at room temperature.
6. Add 10  $\mu\text{L}$  2 M DTT to quench any excess DMA. Centrifuge at  $16,000 \times g$  for 20 minutes at  $4^{\circ}\text{C}$ . Transfer the supernatant to a sterile microcentrifuge tube. The protein concentration of the lysate is ~8–10 mg/mL.
7. Dilute the lysate for each ZOOM<sup>®</sup> Strip as described below. You will need 140  $\mu\text{L}$  of diluted sample per strip. Prepare a master mix of common components for multiple samples.

1.1X ZOOM <sup>®</sup> 2D Protein Solubilizer 1 or 2	128 $\mu\text{L}$
Lysate	10–12 $\mu\text{L}$
2 M DTT	0.7 $\mu\text{L}$
Appropriate ZOOM <sup>®</sup> Carrier Ampholytes (v/v)	0.4–1.6 $\mu\text{L}$ (0.25–1.0%)*
Bromophenol Blue	Trace
Deionized water	to 140 $\mu\text{L}$

\*Use 0.8  $\mu\text{L}$  (0.5%) of ampholytes as a general starting point, except use 0.4  $\mu\text{L}$  (0.25%) of pH 9–11 ampholytes.

8. Proceed to **Rehydrating ZOOM<sup>®</sup> Strips** on page 20.
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## Preparing Samples, continued

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### Preparing Plant Samples

The following procedure uses components of the ZOOM<sup>®</sup> 2D Protein Solubilizer Kit to prepare plant samples for 2D analysis.

1. 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.
2. To 100 mg of ground tissue, add ~1 mL of cold 10% (w/v) TCA, 0.07% (v/v)  $\beta$ -mercaptoethanol acetone, vortex for 1 minute, and then incubate at  $-20^{\circ}\text{C}$  for at least 2 hours. Proceed to Step 5, below.
3. If your starting material is **isolated, pelleted chloroplasts**, resuspend chloroplasts in iso-osmotic solution (0.33 M sorbitol). Centrifuge at  $10,000 \times g$  for 5 minutes at  $4^{\circ}\text{C}$  to pellet chloroplasts. Discard the supernatant and repeat the wash step once.
4. Determine the mass of pelleted chloroplasts. To 50 mg chloroplasts, add ~1 mL cold 10% (w/v) TCA, 0.07% (v/v)  $\beta$ -mercaptoethanol in acetone. Vortex for 1 minute to resuspend the pellet and incubate at  $-20^{\circ}\text{C}$  for at least 2 hours.
5. Centrifuge the precipitated proteins at maximum speed for 20 minutes at  $4^{\circ}\text{C}$  in a microcentrifuge. Discard the supernatant.
6. Resuspend the pellet in 1 mL **cold** acetone containing 0.07% (v/v)  $\beta$ -mercaptoethanol by vortexing. Centrifuge at maximum speed for 20 minutes at  $4^{\circ}\text{C}$  in a microcentrifuge. Discard the supernatant. Repeat washing steps until the pellet is colorless.
7. Discard final supernatant and dry the pellet under vacuum for 2–5 minutes.
8. Resuspend pellet in 950  $\mu\text{L}$  freshly prepared Lysis Buffer from Step 4, previous page.
9. 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.
10. Incubate the lysate on a rotary shaker for 10–15 minutes at room temperature.
11. Add 5  $\mu\text{L}$  99% DMA to the lysate for alkylation. Incubate the lysate on a rotary shaker for 30 minutes at room temperature.
12. Add 10  $\mu\text{L}$  2 M DTT to quench any excess DMA. Centrifuge at  $16,000 \times g$  for 20 minutes at  $4^{\circ}\text{C}$ .
13. Transfer the supernatant containing the lysate to a sterile microcentrifuge tube. At this point you can aliquot the supernatant into smaller volumes and store at  $-85^{\circ}\text{C}$  to  $-68^{\circ}\text{C}$ .

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*Continued on next page*

## Preparing Samples, continued

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### Preparing Plant Samples, continued

14. Dilute the lysate as described below. You need 140  $\mu\text{L}$  of diluted sample per ZOOM<sup>®</sup> Strip.

1.1X ZOOM <sup>®</sup> 2D Protein Solubilizer 1 or 2	128 $\mu\text{L}$
Lysate	10–12 $\mu\text{L}$
2 M DTT	0.7 $\mu\text{L}$
Appropriate ZOOM <sup>®</sup> Carrier Ampholytes (v/v)	0.4–1.6 $\mu\text{L}$ (0.25–1.0%)*
Bromophenol Blue	Trace
Deionized water	to 140 $\mu\text{L}$

\*Use 0.8  $\mu\text{L}$  (0.5%) of ampholytes as a general starting point, except use 0.4  $\mu\text{L}$  (0.25%) for pH 9–11 ampholytes.

15. Proceed to **Rehydrating ZOOM<sup>®</sup> Strips** on page 20.

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### Using Samples from Solution-Phase IEF

You can use fractionated samples from the ZOOM<sup>®</sup> IEF Fractionator with the ZOOM<sup>®</sup> IPGRunner<sup>™</sup> System. You need 140  $\mu\text{L}$  of fractionated sample per ZOOM<sup>®</sup> Strip.

To 140  $\mu\text{L}$  of the fractionated sample, add a trace amount of bromophenol blue dye, and proceed directly to **Rehydrating ZOOM<sup>®</sup> Strips** on page 20.

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# Rehydrating ZOOM® Strips

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## Introduction

Rehydrating ZOOM® Strips in sample rehydration buffer is the first step in isoelectric focusing. The protein sample is added to the sample rehydration buffer containing urea, detergent, reducing agent, ampholyte solution, and a dye. The rehydration buffer denatures and solubilizes the sample proteins for isoelectric focusing.

Each ZOOM® IPGRunner™ Cassette is specifically designed to rehydrate up to six 7.0 cm ZOOM® Strips and perform isoelectric focusing of the ZOOM® Strips in one unit. This eliminates the need to move the ZOOM® Strips from one unit to another for rehydration and isoelectric focusing. The ZOOM® Strips are rehydrated in the ZOOM® IPGRunner™ Cassette by passive rehydration.

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- Always wear gloves while handling the ZOOM® Strips and ZOOM® IPGRunner™ Mini-Cell.
  - To avoid possible contamination from skin-borne proteins, do not touch the ZOOM® Strips with bare hands. Use forceps while handling the ZOOM® Strips and the Electrode Wicks.
- 

## Materials Needed

- Protein sample in sample rehydration buffer for IEF (see pages 14–18 for example sample preparation procedures; see pages 6–11 for sample preparation guidelines)
  - ZOOM® Strips (supplied in the Combo Kit)
  - ZOOM® IPGRunner™ Cassettes (supplied in the Combo Kit)
  - Sealing Tape (supplied in the Combo Kit)
- 

## Note

- You need 140 µL of sample diluted in sample rehydration buffer to rehydrate each ZOOM® Strip for the standard rehydration time of 1 hour. If you want to extend the rehydration time to overnight, you will need 155 µL of diluted sample.
  - You can load IPG strips containing samples with different salt or protein concentrations in the same ZOOM® IPGRunner™ Cassette and perform IEF as described on page 26. However, you may need to optimize the run time based on initial results.
  - Load only one type of strip per cassette.
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## Rehydrating ZOOM® Strips, continued

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### Procedure

Follow the procedure below to rehydrate your ZOOM® Strips:

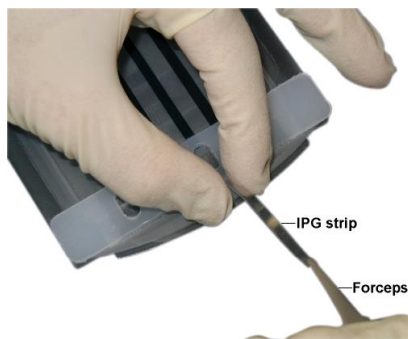
1. Remove the ZOOM® IPGRunner™ Cassette from the box.
2. Set the ZOOM® IPGRunner™ Cassette on a level surface with the Sample Loading Wells facing upwards.
3. Load 140 µL of the sample rehydration buffer containing the protein sample into each Sample Loading Well located at the rounded edge of the ZOOM® IPGRunner™ Cassette (as shown).



**Note:** You do not need to use all the channels. You can run 2 to 6 ZOOM® Strips per ZOOM® IPGRunner™ Cassette. Leave unused channels empty.

4. Remove the ZOOM® Strip card from its pouch and peel a strip away from the card backing. The protective strip cover will remain on the card.
5. Each strip has a gel side and a side with printed markings on it (see page 5). Hold the ZOOM® Strip at the basic end (-) using forceps with the printed side facing *down* (i.e., gel side up). Using your fingers to guide the strip, gently slide the acidic end (+) of the strip into the Enclosed Channel through the Sample Loading Well at the curved end of the ZOOM® IPGRunner™ Cassette. Slide the strip until the acidic end (+) of the strip touches the end of the channel slot (as shown in the following picture).

Avoid introducing large air bubbles while sliding the ZOOM® Strip into the sample well. To remove any large bubbles, slide the strip gently back past the bubble, then back in. Small bubbles will not affect rehydration.



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*Continued on next page*

## Rehydrating ZOOM<sup>®</sup> Strips, continued

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### Procedure, continued

6. Repeat Steps 4 and 5 for the remaining wells containing sample.
7. Seal all Sample Loading Wells (including unused wells) with the Sealing Tape provided in the kit. Make sure that the tape fully seals all wells. Smooth the tape down with a tube or pipette to create a sealed environment for rehydration.

The unique design of the ZOOM<sup>®</sup> IPGRunner<sup>™</sup> Cassette allows oil-free rehydration of ZOOM<sup>®</sup> Strips.

8. Incubate the ZOOM<sup>®</sup> IPGRunner<sup>™</sup> Cassette with ZOOM<sup>®</sup> Strips for **1 hour** at room temperature to rehydrate the strips. You can extend the rehydration time to 2 hours or overnight without any adverse affects. Note that you need to use 155  $\mu$ L of sample rehydration buffer for overnight rehydration.
  9. Proceed to **Assembling the ZOOM<sup>®</sup> IPGRunner<sup>™</sup> for Focusing**, page 23.
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# Assembling the ZOOM® IPGRunner™ for Focusing

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## Introduction

After rehydrating your ZOOM® Strips, you are ready to perform isoelectric focusing. The ZOOM® IPGRunner™ Core is specifically designed to fit into the ZOOM® IPGRunner™ Mini-Cell and can only be used with this apparatus.

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## Materials Needed

- Deionized water
  - Filter paper
  - Electrode Wicks (included in the Combo kit)
  - ZOOM® IPGRunner™ Mini-Cell (included in the Combo kit)
- 

## Applying Electrode Wicks

Electrode wicks serve as an electrophoresis reservoir, help to remove salts and other charged molecules from the sample, and prevent accumulation of charged molecules at the electrodes.

1. Remove the Sealing Tape and two Sample Loading Devices from the ZOOM® IPGRunner™ Cassette. The adhesive will be exposed on the Film Cover. Verify that portions of the gel are exposed at the anodic and cathodic ends of the Cassette. You may adjust gel to the correct position using forceps.
  2. Place an Electrode Wick at each end of the ZOOM® IPGRunner™ Cassette over the adhesive. Use the black alignment marks on the cassette to properly place the wicks. Apply gentle pressure to secure the Electrode Wicks on to the ZOOM® IPGRunner™ Cassette.
  3. Evenly apply 600 µL of deionized water to each of the Electrode Wicks. **Do not use ultrapure water.**
  4. Assemble the ZOOM® IPGRunner™ and perform IEF as described on page 24.
- 

*Continued on next page*

## Assembling the ZOOM® IPGRunner™ for Focusing, continued

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### Assembling ZOOM® IPGRunner™

Instructions are provided below to assemble the ZOOM® IPGRunner™ Cassette containing the rehydrated ZOOM® Strips and ZOOM® IPGRunner™ Buffer Core:

1. Set the ZOOM® IPGRunner™ Core and ZOOM® IPGRunner™ Cassette with the rehydrated strips on a flat surface in an upright position.
2. Slide the ZOOM® IPGRunner™ Cassette towards the electrodes of the ZOOM® IPGRunner™ Core until the Electrode Wicks of the ZOOM® IPGRunner™ Cassette are in contact with the electrodes of the ZOOM® IPGRunner™ Core (see figure below). Hold the ZOOM® IPGRunner™ Cassette/ZOOM® IPGRunner™ Core sandwich with your hands.



3. If you are using two ZOOM® IPGRunner™ Cassettes, repeat Step 2 with a second ZOOM® IPGRunner™ Cassette.

If you are using only one ZOOM® IPGRunner™ Cassette, use the Buffer Dam in place of the second cassette.

4. While holding the sandwich together with your hands (see figure below), slide the sandwich containing the ZOOM® IPGRunner™ Core and ZOOM® IPGRunner™ Cassettes (or Buffer Dam) into the Mini-Cell Chamber of the ZOOM® IPGRunner™ System. The negative electrode must fit into the opening in the gold plate on the Mini-Cell Chamber.



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*Continued on next page*



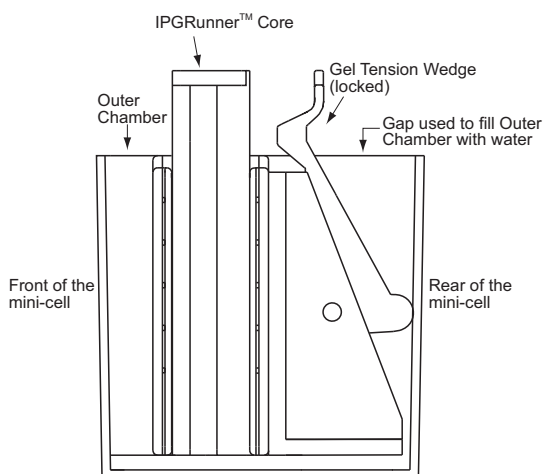
## Assembling the ZOOM® IPGRunner™ for Focusing, continued



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### Assembling ZOOM® IPGRunner™, continued

5. Insert the Gel Tension Wedge into the Mini-Cell behind the ZOOM® IPGRunner™ Core. Make sure that the Gel Tension Wedge is in its unlocked position, allowing the wedge to slide easily into the Mini-Cell. The Gel Tension Wedge should rest on the bottom of the Mini-Cell Chamber (see the following figure).
6. Pull Gel Tension lever toward the front of the ZOOM® IPGRunner™ Mini-Cell until the Lever comes to a firm stop and the ZOOM® IPGRunner™ Cassettes or ZOOM® IPGRunner™ Cassette/Buffer Dam are pressed snugly against the ZOOM® IPGRunner™ Core (see the following figure).
7. Fill Outer Chamber with 600 mL of deionized water by pouring the water through the gap between the Gel Tension Wedge and back of the Outer Chamber (see the following figure). Take care that the water does not spill into the Inner Chamber.

**Caution: Do not pour water or liquid into the Inner Chamber of the Mini-Cell.**



-  Do not handle the lid if the cables are plugged into the power supply.
  8. Place the ZOOM® IPGRunner™ Cell Lid on the ZOOM® IPGRunner™ Core. The lid can only be firmly seated if the (-) electrode is aligned over the banana plug on the right. If the lid is not positioned properly, no power will flow through the ZOOM® IPGRunner™ System.
  9. With the power supply turned **off**, connect electrode cords to the power supply [red to (+) jack, black to (-) jack].
  -  Make sure that the power is turned off before connecting the ZOOM® IPGRunner™ Mini-Cell to the power supply.
  10. Turn on the power. See page 26 for **Performing IEF**.
-

# Performing IEF

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## Introduction

Isoelectric focusing of the ZOOM® Strips is performed at low current. You need a power supply capable of operating at very low current (see page 13).

To obtain the best results, IEF is typically performed by increasing the voltage gradually (using a voltage ramp or in steps) and maintaining the final focusing voltage for 30–100 minutes.

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## Important

The ZOOM® IPGRunner™ System is designed to operate and perform optimally at a voltage much lower than recommended for other IEF systems. You will obtain good focusing using conditions described on the next page for most protein samples. **Do not** operate the ZOOM® IPGRunner™ System at a very high voltage as recommended for conventional IEF methods.

---

## Choosing an Electrofocusing Protocol

Based on the capability of your power supply, you need to choose an appropriate electrofocusing protocol, A or B. Electrofocusing protocols A and B are provided on the next page.

**Note:** See the ZOOM® IEF Fractionator manual for electrofocusing protocols for fractionated samples.

The table below allows you to choose an appropriate protocol based on the capability of your power supply:

If Your Power Supply has....	Then Choose....
Voltage ramp capability, Current limit capability, and Power limit capability	Protocol A
Step voltage capability, Current limit capability, and Power limit capability	Protocol B
Step voltage capability only	Protocol B
Voltage ramp capability only	Protocol A

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*Continued on next page*

## Performing IEF, continued

### Electrophoresis Conditions

Choose the appropriate electrofocusing protocol based on the capability of your power supply (see table on the previous page). You can vary the time and voltage of IEF depending on the amount and nature of your protein sample.

**Note:** The ZOOM® IPGRunner™ System is rated to 3,500 VDC and 3.5 Watts, but for performing IEF the maximum recommended voltage is 2000 V and 0.1 W per strip (~50 µA per strip).

If the power supply has a current and power limiting capability, we recommend setting a current limit at 50 µA per strip and a power limit at 0.1 W per strip.

1. Perform IEF using an appropriate protocol (A or B). See the ZOOM® IEF Fractionator manual for electrofocusing protocols for fractionated samples.

Broad Range ZOOM® Strips	Narrow Range ZOOM® Strips	ZOOM® Strips pH 9–12
<i>Protocol A (Voltage Ramp)</i>	<i>Protocol A (Voltage Ramp)</i>	<i>Protocol A (Voltage Ramp)</i>
175 V for 15 minutes 175–2000 V ramp for 45 minutes 2000 V for 30 minutes	175 V for 15 minutes 175–2000 V ramp for 45 minutes 2000 V for 105 minutes	175 V for 15 minutes 175–2000 V ramp for 45 minutes 2000 V for 60 minutes
<b>OR</b>	<b>OR</b>	<b>OR</b>
<i>Protocol B (Step Voltage)</i>	<i>Protocol B (Step Voltage)</i>	<i>Protocol B (Step Voltage)</i>
200 V for 20 minutes 450 V for 15 minutes 750 V for 15 minutes 2000 V for 30 minutes	200 V for 20 minutes 450 V for 15 minutes 750 V for 15 minutes 2000 V for 105 minutes	200 V for 20 minutes 450 V for 15 minutes 750 V for 15 minutes 2000 V for 60 minutes

**Tip:** If current is flowing through the system, the bromophenol blue tracking dye will move towards the anode.

**Note:** You may need to focus ZOOM® Strips, pH 6–10 for ~30 minutes longer at 2000 V, depending on your type of sample. Crude protein mixtures, high protein loads, or samples containing high salt concentrations (>10 mM) may require longer run times or more total volt hours for optimal resolution. Based on your initial 2D result, you may increase run time of the final focusing step in increments of 15 minutes to optimize IEF separation.

2. At the end of the run, turn off the power, disconnect cables from the power supply, and proceed to **Disassembling the ZOOM® IPGRunner™**, page 28.



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

### Volt-Hours

The resultant final volt-hour for isoelectric focusing of protein samples using the ZOOM® IPGRunner™ is 1,200–4,200 Vh.

# Disassembling the ZOOM<sup>®</sup> IPGRunner<sup>™</sup>

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## Disassembling the ZOOM<sup>®</sup> IPGRunner<sup>™</sup>



Be sure the cables are disconnected from the power supply.

1. Remove the lid.
2. Pour off water from the Outer Chamber.
3. Unlock the Gel Tension Wedge and remove the wedge from the ZOOM<sup>®</sup> IPGRunner<sup>™</sup> Mini-Cell.
4. Remove the ZOOM<sup>®</sup> IPGRunner<sup>™</sup> Core/ZOOM<sup>®</sup> IPGRunner<sup>™</sup> Cassette sandwich assembly from the Mini-Cell Chamber.
5. Remove the ZOOM<sup>®</sup> IPGRunner<sup>™</sup> Cassettes and lay cassettes on a flat surface, such as the bench top.

Proceed to **Performing 2D Electrophoresis Using ZOOM<sup>®</sup> Gels**, page 29, or **Staining ZOOM<sup>®</sup> Strips**, page 43.

**Note:** You can store the entire cassette in a sealed container at  $-80^{\circ}\text{C}$  until you are ready to use the strips.

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## Removing ZOOM<sup>®</sup> Strips from the Cassette

**Note:** Do not remove the ZOOM<sup>®</sup> Strips from the cassette if you are using the ZOOM<sup>®</sup> Equilibration Tray for 2D electrophoresis (page 29) or staining the strips (page 43).

To remove the strips from the cassette.

1. Firmly hold the ZOOM<sup>®</sup> IPGRunner<sup>™</sup> Cassette and peel off the film cover. Start peeling the film from each top edge of the cassette and pull towards the bottom of the cassette.
2. Remove ZOOM<sup>®</sup> Strips from the ZOOM<sup>®</sup> IPGRunner<sup>™</sup> Cassette using forceps.

You can store ZOOM<sup>®</sup> Strips in a sealed container at  $-80^{\circ}\text{C}$ .

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## Note

The ZOOM<sup>®</sup> IPGRunner<sup>™</sup> Cassettes are disposable. After use, discard the cassette. Do not re-use the ZOOM<sup>®</sup> IPGRunner<sup>™</sup> Cassettes.

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## Maintenance

Wash the ZOOM<sup>®</sup> IPGRunner<sup>™</sup> Mini-Cell with a mild detergent and rinse with deionized water after each use. **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<sup>®</sup> IPGRunner<sup>™</sup> System.

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# Performing SDS-PAGE Using ZOOM® Gels

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## Introduction

After you have completed isoelectric focusing of your protein, you are ready to perform second dimension electrophoresis using SDS-PAGE. This section provides a protocol for 2D electrophoresis using ZOOM® Gels and the XCell SureLock® Mini-Cell available from Life Technologies.

If you are using any other gel electrophoresis system, refer to the manufacturer's recommendations. If you are not using pre-cast gels, see the following **Note** this page.

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## 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. Two types of ZOOM® Gels are available separately (see page 55 for ordering information).

- NuPAGE® Novex® 4–12% Bis-Tris ZOOM® Gel
  - Novex® 4–20% Tris-Glycine ZOOM® Gel
- 

## Important

ZOOM® Strips are not compatible with 2D well gels. 2D well gels are designed for tube gels, not IPG strips.

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## Note

If you are preparing your own SDS gel, prepare the gel before you begin the equilibration step. Refer to *Current Protocols in Molecular Biology* (Ausubel et al., 1994) for information on preparing SDS polyacrylamide gels and performing SDS-PAGE.

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## Second Dimension Electrophoresis

The 2D electrophoresis procedure involves reducing and alkylating the proteins focused on the ZOOM® Strip in equilibration buffer, loading the strip on a second dimension gel, and performing SDS-PAGE.

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## Materials Needed

See page 55 for ordering information.

- 4X NuPAGE® LDS Sample Buffer
  - NuPAGE® Sample Reducing Agent
  - NuPAGE® Antioxidant
  - ZOOM® Equilibration Tray
  - ZOOM® Gel
  - Appropriate running buffer depending on the type of gel you are using
  - 0.5% agarose solution in running buffer
  - Plastic flexible ruler or thin weighing spatula
  - Water bath set at 55°C or 65°C
  - XCell SureLock® Mini-Cell
  - Protein molecular weight marker (Mark12™ Unstained Standard)
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*Continued on next page*

## Performing SDS-PAGE Using ZOOM® Gels, continued

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### ZOOM® Equilibration Tray

The ZOOM® Equilibration Tray is a convenient, disposable tray designed for use with ZOOM® IPGRunner™ Cassettes to perform equilibration of ZOOM® Strips prior to second dimension SDS-PAGE. The tray contains an adhesive on the inner side. After removing the adhesive liner from the tray and placing the tray on a dry surface of the ZOOM® IPGRunner™ Cassette, the adhesive creates a tight seal between the tray and ZOOM® IPGRunner™ Cassette and holds each ZOOM® Strip in the individual channels of the cassette.

For ordering information, see page 55. The ZOOM® Equilibration Tray manual is available at [www.lifetechnologies.com](http://www.lifetechnologies.com).

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### Preparing Buffers for Equilibration

Incubating ZOOM® Strips in NuPAGE® LDS Sample Buffer equilibrates the strips in SDS buffer and prepares the strips for 2D SDS-PAGE. We recommend using the NuPAGE® LDS Sample Buffer with NuPAGE® Novex® 4–12% Bis-Tris ZOOM® Gels or Novex® 4–20% Tris-Glycine ZOOM® Gels. **You need 5–15 mL of buffer per equilibration tray.**

#### For equilibration

1. Dilute 4X NuPAGE® LDS Sample Buffer to 1X with deionized water.
2. Add 1.0 mL NuPAGE® Sample Reducing Agent (10X) to 9.0 mL 1X NuPAGE® LDS Sample Buffer from Step 1 in a 15-mL conical tube.

#### For alkylation

Prepare 125 mM Alkylating Solution by dissolving 232 mg of fresh iodoacetamide in 10 mL of 1X NuPAGE® LDS Sample Buffer (from Step 1, above) in a 15-mL conical tube.

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### Attaching the ZOOM® Equilibration Tray

After you have disassembled the ZOOM® IPGRunner™ (Step 6, page 28), follow the steps below:

1. Blot any excess liquid from the ZOOM® IPGRunner™ Cassette using a paper towel and ensure your hands are dry.
2. Firmly hold the ZOOM® IPGRunner™ Cassette and peel off the film cover from the cassette with your dry hands. Avoid introducing any liquid on the cassette surface.

**Note:** You may equilibrate less than 6 strips using the tray. Remove the desired quantity of strips from the cassette and store the strips in a sealed container at –80°C. When you are ready to equilibrate, place the strips in a cassette and proceed to equilibration as described page 31.

3. Remove one ZOOM® Equilibration Tray from the package and pull on the adhesive liner tab to remove the liner.
  4. Hold the ZOOM® Equilibration Tray (adhesive side down) and align the tray between the two ribs (protrusions) of the cassette such that the Life Technologies logo is towards the flat end of the cassette (see figure A, page 31). **Place the tray on the cassette and apply firm pressure to the tray on the adhesive area with the blunt end of a pen to ensure a tight seal between the tray and cassette (figure B, next page).** A tight seal is formed when the adhesive changes from a cloudy to clear appearance.
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*Continued on next page*

## Performing SDS-PAGE Using ZOOM® Gels, continued

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### Figures

Figure A. Aligning the Tray on the Cassette

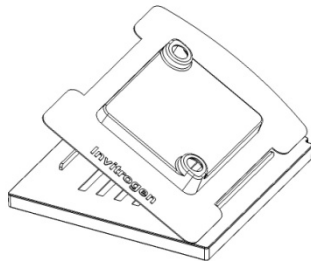
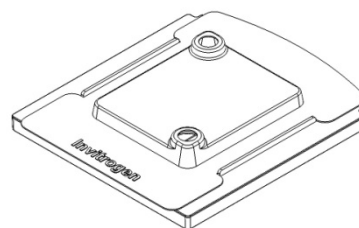


Figure B. Tray Attached to the Cassette



### Important

- It is important to create a tight seal between the ZOOM® Equilibration Tray and ZOOM® IPGRunner™ Cassette to prevent leaks. To obtain a tight seal, we recommend pressing the tray firmly on the cassette using the blunt end of a pen. A tight seal is formed when the adhesive changes from a cloudy to clear appearance.
  - To ensure a tight seal, avoid introducing any liquid in the cassette area where the ZOOM® Equilibration Tray will be placed. If necessary, remove any remaining large pieces of adhesive after removing the Film Cover from the ZOOM® IPGRunner™ Cassette.
- 

### Equilibrating the ZOOM® Strip

1. Add 5–15 mL 1X NuPAGE® LDS Sample Buffer with reducing agent (see **Preparing Buffers for Equilibration**, Step 2, page 30) through either spout on the tray. If you notice leaking into the adhesive area between the tray and the cassette, check the seal and verify that you are not using more than 15 mL of buffer with reducing agent.
  2. Incubate for 15 minutes on a rotary shaker or benchtop.
  3. Decant buffer into a waste container using the spouts on the tray.
  4. Add 5–15 mL Alkylating Solution (**Preparing Buffers for Equilibration**, page 30) through either spout on the tray. Incubate for 15 minutes on a rotary shaker or benchtop.
  5. Decant buffer into a waste container using the spouts on the tray. Shake out any residual buffer.
  6. Remove the ZOOM® Equilibration Tray from the cassette by first pulling on the corners of the tray and then firmly remove the tray. If desired, you may use a gel knife.
  7. Remove the equilibrated ZOOM® Strips from the cassette using forceps and immediately proceed to **Applying IPG Strips**, page 32.
- 

### Note

You may use NuPAGE® Novex® 4–12% Bis-Tris ZOOM® Gel, Novex® 4–20% Tris-Glycine ZOOM® Gel, or any other SDS/PAGE gel of choice for performing second dimension SDS/PAGE. Use an appropriate percentage of acrylamide gel that will best resolve your proteins of interest.

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*Continued on next page*

## Performing SDS-PAGE Using ZOOM® Gels, continued

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### Applying IPG Strips

Apply the equilibrated ZOOM® Strip to the second dimension SDS gel as described below:

1. Prepare 0.5% agarose solution in the appropriate running buffer and keep it warm (55°C to 65°C) until you are ready to use the agarose solution. You will need ~400 µL of 0.5% agarose solution for each ZOOM® Gel.
  2. Remove the ZOOM® gel cassette from the pouch and peel off the tape covering the slot on the back of the gel cassette.
  3. If the molecular weight marker well in the ZOOM® gel is bent, straighten the well using a gel loading tip.
  4. Cut plastic ends of the IPG strip flush with the gel. Do not cut off any portions of the gel.
  5. Slide the IPG strip into the ZOOM® Gel well. Be sure that the gel side of the strip faces the front of the cassette.
  6. Align the IPG strip properly in the ZOOM® Gel well using a thin plastic ruler or a weighing spatula. Avoid introducing any air bubbles while sliding the strip.
  7. Add ~400 µL of 0.5% agarose solution from Step 1 into the ZOOM® Gel well containing the IPG strip. Take care that the agarose solution does not overflow into the molecular weight marker well. Allow the agarose solution to solidify.
  8. Proceed to SDS-PAGE, immediately following.
- 

### SDS-PAGE

The following procedure uses ZOOM® Gels with the XCell *SureLock*® Mini-Cell. Download the XCell *SureLock*® Mini-Cell manual from [www.lifetechnologies.com](http://www.lifetechnologies.com). If you are using any other electrophoresis system, refer to the manufacturer's recommendations.

1. Assemble the gel cassette/Buffer Core sandwich as described in the XCell *SureLock*® Mini-Cell manual. If you are using only one ZOOM® gel, use the Buffer Dam to replace the second gel cassette.  
**Note:** Do not use the ZOOM® IPGRunner™ Core for electrophoresis of the second dimension gel. You must use the Buffer Core supplied with the XCell *SureLock*® Mini-Cell.
  2. Fill the Lower Buffer Chamber and Upper Buffer Chamber with the recommended volume of an appropriate running buffer. Add 0.5 mL of NuPAGE® Antioxidant to the Upper Buffer Chamber.
  3. Load molecular weight standards in the marker well.
  4. Place the XCell *SureLock*® Mini-Cell lid on the Buffer Core. With the power on the power supply turned off, connect the electrode cords to the power supply [red to (+) jack, black to (-) jack].
  5. Perform SDS-PAGE at 200 V for 40–50 minutes for NuPAGE® Novex® Bis-Tris ZOOM® Gel or at 125 V for 90 minutes for Novex® Tris-Glycine ZOOM® Gel.
  6. At the end of electrophoresis, turn off the power and disassemble the gel cassette/Buffer Core sandwich assembly as described in the XCell *SureLock*® Mini-Cell manual.
  7. Proceed to gel staining and/or mass spectrometry analysis.
-


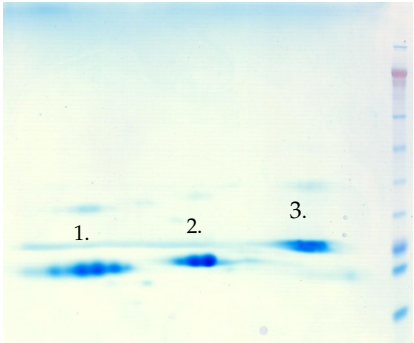
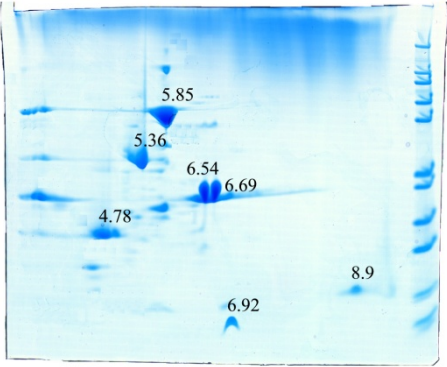


# Expected Results

## Introduction





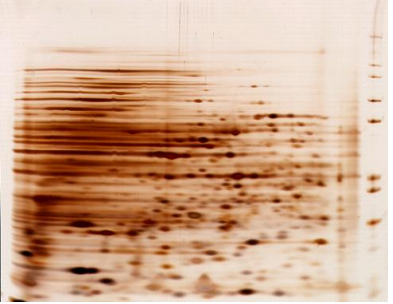

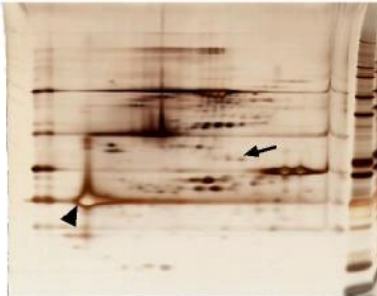
Examples of expected results of 2D gel electrophoresis performed under various conditions with the ZOOM® IPGRunner™ are shown below.

First dimension IEF was performed on ZOOM® Strips using the ZOOM® IPGRunner™ System as described in this manual. Second dimension SDS-PAGE was performed on NuPAGE® Novex® 4–12% Bis-Tris ZOOM® Gel with XCell SureLock® Mini-Cell using standard conditions. Each gel was stained with SilverQuest™ Silver Staining Kit or SimplyBlue™ SafeStain. The protein marker used on all gels is Mark12™ Unstained Standard.

 <p>pH 4                      pH 7</p>	<p><b>ZOOM® Strip pH 4–7</b></p> <p>First dimension IEF of <i>E. coli</i> lysate (~5 µg) was performed on ZOOM® Strip pH 4–7. The resulting 2D gel was silver stained.</p>
 <p>1.                      2.                      3.</p> <p>pH 9                      pH 12</p>	<p><b>ZOOM® Strip pH 9–12</b></p> <p>First dimension IEF of a set of basic proteins,</p> <ol style="list-style-type: none"><li>1. Cytochrome C, pI 9.5–10.0;</li><li>2. Lysozyme, pI 10.7–10.9;</li><li>3. Histone H3, pI 11.8)</li></ol> <p>was performed on ZOOM® Strip pH 9–12. The resulting 2D gel was stained with SimplyBlue™ SafeStain.</p>
 <p>5.85 5.36 6.54 6.69 4.78 6.92 8.9</p> <p>pH 3                      pH 10</p>	<p><b>ZOOM® Strip pH 3–10L (linear)</b></p> <p>First dimension IEF of a set of proteins was performed on ZOOM® Strip pH 3–10L. The resulting 2D gel was stained with SimplyBlue™ SafeStain.</p>

Continued on next page

## Expected Results, continued

<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>10 mM NaCl</p>  </div> <div style="text-align: center;"> <p>20 mM NaCl</p>  </div> </div>	<p><b>Salt Concentration Too High</b></p> <p><i>E. coli</i> lysate was subjected to 2D electrophoresis in the presence of salt. The gel was silver stained. As seen in the figure, increasing the salt concentration results in horizontal streaking.</p>
<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p><b>A</b></p>  </div> <div style="text-align: center;"> <p><b>B</b></p>  </div> </div>	<p><b>Underfocusing</b></p> <p><i>E. coli</i> lysate was subjected to IEF under different conditions and silver stained.</p> <p><b>A:</b> 765 volt-hours, underfocused  <b>B:</b> 1,300 volt-hours, focused</p> <p>Underfocusing results in streaking and poor resolution of spots. As seen in Figure A, the proteins require more time to focus. Increasing the volt-hours (time), improves resolution (Figure B).</p>
	<p><b>Overloading</b></p> <p><i>E. coli</i> lysate (80 <math>\mu</math>g) was subjected to 2D electrophoresis and silver stained. The resolution is lost when proteins are overloaded as seen in the figure. The recommended protein load for silver staining is 5–15 <math>\mu</math>g.</p>
<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p><b>A</b></p>  </div> <div style="text-align: center;"> <p><b>B</b></p>  </div> </div>	<p><b>Low abundance proteins</b></p> <p>Protein mixture (10–15 <math>\mu</math>g of 5 purified proteins) was subjected to 2D electrophoresis.</p> <p><b>A:</b> Stained with SimplyBlue™ Safestain  <b>B:</b> Stained with SilverQuest™ Silver Staining Kit</p> <p>The low abundance proteins (contaminants) are only visualized on the silver stained gel (indicated by an arrow). Silver staining a high protein load (40 <math>\mu</math>g) is necessary to visualize low abundance proteins while abundant proteins produce dark or negatively silver stained spots (indicated by a closed arrowhead).</p>

# Troubleshooting

## Introduction

Review the information below to troubleshoot your experiments. To troubleshoot SDS-PAGE using the XCell *SureLock*<sup>®</sup> Mini-Cell, refer to the XCell *SureLock*<sup>®</sup> Mini-Cell manual (IM-9003) available at [www.lifetechnologies.com/manuals](http://www.lifetechnologies.com/manuals).

Observation	Cause	Solution
No current for isoelectric focusing	Poor contact between electrodes or incomplete circuit	Make sure that you have added 600 $\mu$ L deionized water to the Electrode Wicks and the gel is exposed at the anodic and cathodic ends of the cassette. Check the power supply. Be sure to set the 'Load Check' to off to enable the power supply to operate at low current.
Low current or the current read out is 0 mA	It is normal for IEF to proceed at a low current ( $\mu$ A range)	Use proper power supply as recommended on page 13.
No current or low current at the start of SDS/PAGE	Incorrect buffers used	Prepare fresh buffers using the correct recipe.
	Buffer reservoir not filled properly	Fill the reservoirs with the recommended volumes.
No distinct spots	Low protein load	Increase the protein load. Use an accurate and sensitive protein estimation method.
	Improper sample preparation	Increase solubilization reagents. Use at least 8 M urea for solubilization. Add DTT and non-ionic detergents (see page 8 for details).
	Strip not correctly oriented	Align the strip correctly as described on page 20. Be sure to have the gel side up when loading the strip into the ZOOM <sup>®</sup> IPGRunner <sup>™</sup> Cassette.
	Air bubbles between the strip and 2D gel	Smooth out any air bubbles.
	Insensitive detection method	Use sensitive detection methods such as silver staining or immunoblotting.
Burning of strips (arcing)	High salt concentration	Limit the salt concentration in the samples to 10 mM or less. If possible, adjust the salt concentration of your sample by ultrafiltration, dialysis, or gel filtration.
	High Power	Check power settings.
	Poor strip rehydration	Rehydrate the strips in 140 $\mu$ L sample rehydration buffer for 1 hour as described on page 21. Rehydration can be extended to overnight if you use 155 $\mu$ L rehydrating buffer. Make sure the rehydration buffer is covering the strip completely.
	Liquid in the inner chamber	<b>Do not</b> pour any liquid or buffer in the inner chamber. Check for any leakage in the inner chamber.

*Continued on next page*

## Troubleshooting, continued

Observation	Cause	Solution
Incomplete focusing	Improper sample preparation	Increase solubilization reagents in the rehydration buffer. Use 8 M urea for solubilization. Add DTT and non-ionic detergents. See page 8 for more details.
	Incorrect focusing time	Increase or decrease the focusing time based on the initial results.
	High protein load	Decrease the protein load. Use an accurate and sensitive protein estimation method.
	Salt, lipids, or nucleic acid impurities present in the sample	Remove interfering substances (see page 10).
	Poor strip rehydration	Rehydrate the strips in 140 $\mu$ L rehydrating buffer for 1 hour as described on page 21. Rehydration can be extended to overnight if you use 155 $\mu$ L rehydrating buffer. Make sure the rehydration buffer is covering the strip completely.
Horizontal streaking	Impure solutions	Use ultra pure reagents to prepare the rehydration buffer, equilibration buffer, and buffers for SDS/PAGE.
	Air bubble between the strip and 2D gel	Smooth out the air bubbles.
	Poor strip rehydration	Rehydrate the strips in 140 $\mu$ L rehydrating buffer for 1 hour as described on page 21. Rehydration can be extended to overnight if you use 155 $\mu$ L rehydrating buffer. Make sure the rehydration buffer covers the strip completely.
	Protein overload	Decrease the protein concentration or lower the sample volume.
	Protein precipitates	Increase solubilization reagents in the rehydration buffer (see page 8). Use appropriate strips based on the pI of the protein sample. Do not add more than 10 $\mu$ L of your sample to 140 $\mu$ L of rehydration buffer (see page 14)
Vertical streaking	Impure solutions	Use ultra pure reagents to prepare the rehydration buffer, equilibration buffer, and buffers for SDS/PAGE.
	Protein oxidized	Include DTT in the rehydration buffer and perform the alkylation step.

*Continued on next page*

## Troubleshooting, continued

Observation	Cause	Solution
Protein spots missing or appear as a smear	Protein degradation	Add protease inhibitors during sample preparation (see page 9).
	Different subunits	Use denaturing conditions (8 M urea).
	Incomplete equilibration	Perform equilibration as described on page 31. Increase the equilibration time.
	Protein precipitates	Increase solubilization reagents in the rehydration buffer (see page 8). Use appropriate strips based on the pI of the protein sample.
	Low protein load	Increase the protein load. You can load up to 400 µg of fractionated protein sample per ZOOM® Strip. Use an accurate and sensitive protein estimation method.
	Insensitive detection method	Use sensitive detection methods such as silver staining or immunoblotting.
Uneven background staining of the 2D gel	Background staining due to ampholytes	Prior to staining the 2D gel, thoroughly wash the gel to remove ampholytes. Use ZOOM® Carrier Ampholytes (see page 57 for ordering information) as they provide a clear background due to very low non-specific binding of dyes and stains.
	Staining protocol modified	Refer to the manufacturer's recommendations for correct staining protocols. To achieve best results, be sure to follow all steps exactly as given in the protocol, especially for silver staining. Changes in the protocol can result in high background.

# Appendix

## Safety Information

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### Informational Symbols



The symbols used on the ZOOM® IPGRunner™ are explained below:

Used on the ZOOM® IPGRunner™ Cell Lid and in the manual to indicate an area where a potential shock hazard may exist.

Used on the ZOOM® IPGRunner™ Cell Lid and in the manual 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.

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### Safety

The ZOOM® IPGRunner™ 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: 3500 VDC\*

Maximum Power: 3.5 Watts\*

The ZOOM® IPGRunner™ 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)

Life Technologies 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 Life Technologies. There are no parts that can be serviced by the user.

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The lid of the ZOOM® IPGRunner™ is designed such that if the lid is removed the electrical connection to the gel 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 use lids from other mini-cells with the ZOOM® IPGRunner™ System**
  - **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 26 for details on performing IEF.

# Sicherheits-Anweisungen

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## Symbolerklärung



Im folgenden Abschnitt sind die Gefahrenhinweissymbole auf dem ZOOM® IPGRunner™ erklärt:

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

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

WEEE

## Sicherheits-Anweisungen

Die ZOOM® IPGRunner™ 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: 3500 Volt\*

Maximale Leistung: 3,5 Watt\*

Die ZOOM® IPGRunner™ entspricht den Sicherheits-Richtlinien EN 61010-1. Es besteht keinerlei Gefährdung für den Benutzer, wenn die Richtlinien der Gebrauchsanweisung eingehalten werden. Wird die Elektrophorese-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).

Life Technologies 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 Life Technologies oder einem von Life Technologies autorisierten Händler vorgenommen werden.



Beim Entfernen des Kammerdeckels der ZOOM® IPGRunner™ wird der Stromfluss unterbrochen. Eine Gefährdung durch Stromschlag besteht aber bei aber noch, solange, das Gerät an die Spannungsquelle angeschlossen ist. Die Benutzung der Zelle ohne einen für das Gerät vorgesehenen Deckel ist verboten.

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\*Die empfohlenen Werte für die optimale Benutzung sind niedriger als die angegebene maximale Spannung / Leistung. Seite 26 enthält genauere Hinweise zur Durchführung von IEF.

# Instructions de securite

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## Signification des symboles



Precaution



Danger



Les symboles utilises sur le ZOOM® IPGRunner™ sont expliques ci-dessous:

Sur le couvercle du ZOOM® IPGRunner™ et dans le manuel pour signaler les zones où un risque de potentiel de choc électrique peut exister.

Sur le ZOOM® IPGRunner™ et dans le manuel pour signaler un danger. Veuillez consulter le mode d'emploi pour eviter tout risqué potentiel pour l'utilisateur ou pour l'équipement.

WEEE

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## Instructions de securite

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

Tension maximum: 3500 V continu\*

Puissance maximum: 3.5 W\*

Le ZOOM® IPGRunner™ satisfait aux normes de sécurité EN 61010-1. La sécurité est assurée dans tous les cas d'une utilisation conforme au manuel d'utilisation. Dans le cas ou 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 Life Technologies ne peut être mise en cause en cas d'accident et dommages causés par une utilisation non conforme de la Cellule d'Electrophorèse. Toute intervention de service ou de réparation doit être uniquement effectuée par Life Technologies ou par un agent agréé de Life Technologies. Aucune partie ne peut être reparee par l'utilisateur.

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Precaution

Le ZOOM® IPGRunner™ est conçue de façon à ce que lorsque le couvercle de la cuve est ouvert, la connection électrique avec l'unité est interrompue, mais un risqué de choc électrique [ersiste tant que les lectrodes sont branches. Il ne faut en aucun cas essayer de faire fonctionner la cuve sans le couvercle.

- Ne pas utiliser le couvercle d'autres sure le a ZOOM® IPGRunner™
  - Ne pas toucher pu nettoyer le couvercle lorsque les cables sont branches
- 

\*Les valeurs recommandées pour une performance optimale sont inférieures au voltage et wattage maximum ci-dessus, voir page 26 pour les conditions de migratin IEF.



# Sample Preparation—Alternative Protocol

## Introduction

This section provides an alternative protocol for preparing samples using the Sample Rehydration Buffer.

We recommend using the ZOOM® 2D Protein Solubilizer Kit to prepare samples, as described starting on page 14.

## Sample Rehydration Buffer (1X)

8 M Urea (see page 55 for ordering information). De-ionize the urea before preparation on a mixed bed ion exchanger resin using manufacturer's recommendations.

2% CHAPS

0.5% (v/v) ZOOM® Carrier Ampholytes (see **Note** and **Recommendation** below)

0.002% Bromophenol Blue

1. To prepare 25 mL of 1X Sample Rehydration Buffer, add the following reagents to 15 mL of deionized water

Urea	12 g
CHAPS	0.5 g
Ampholytes	125 µL (see <b>Note</b> below)
Bromophenol Blue	0.5 mL of a 0.1% solution

2. Bring the volume up to 25 mL. Aliquot the buffer into 1 mL aliquots and store at -20°C.
3. Prior to using this buffer, add dithiothreitol (DTT) to a final concentration of 20 mM.

**Note:** Use the appropriate ZOOM® Carrier Ampholytes based on the ZOOM® Strip as described below:

ZOOM® Strip	ZOOM® Carrier Ampholytes
pH 3–10NL (non-linear)	use 125 µL pH 3–10 ampholytes
pH 3–10L (linear)	use 125 µL pH 3–10 ampholytes
pH 4–7	use 125 µL pH4–7 ampholytes
pH 6–10	use 63 µL pH 6–9 and 63 µL pH 9–11 ampholytes
pH 4.5–5.5	use 125 µL of pH 4–6 ampholytes
pH 5.3–6.3	use 125 µL of pH 5–7 ampholytes
pH 6.1–7.1	use 125 µL of pH 6–8 ampholytes
pH 9–12	use 63 µL of pH 9–11 ampholytes

ZOOM® Carrier Ampholytes are available separately, see page 57 for ordering information.



The recommended ampholyte concentration in the sample rehydration buffer is 0.5%. If you are loading 5–50 µg of protein (pure protein or crude lysate) per ZOOM® strip, use 0.5% ampholytes in the sample rehydration buffer.

If you are loading >50 µg of protein (crude lysate or fractionated sample) per ZOOM® Strip, use 0.5–2% ampholytes in the sample rehydration buffer.

**Note:** Higher ampholyte concentration requires longer focusing times.

*Continued on next page*

## Sample Preparation—Alternative Protocol, continued

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### Sample Preparation Using the Sample Rehydration Buffer

The following sample preparation protocol uses the Sample Rehydration Buffer recipe provided on the previous page. Remove any interfering materials as described on page 10 from your sample.

1. Add DTT to the sample rehydration buffer to a final concentration of 20 mM.
2. To 140  $\mu$ L of sample rehydration buffer (1X), add the appropriate amount of protein sample (see **Amount of Protein**, page 15) in a total volume of 5–10  $\mu$ L.

Avoid adding more than 10  $\mu$ L of your protein sample to 140  $\mu$ L of sample rehydration buffer. Adding more than 10  $\mu$ L of sample to the rehydration buffer will decrease the concentration of urea in the sample rehydration buffer to <7.5 M, resulting in protein precipitation.

3. Mix well and proceed to **Rehydrating ZOOM<sup>®</sup> Strips**, page 20.
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# Staining ZOOM® Strips

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## Note

We do not recommend using stained ZOOM® Strips in 2D electrophoresis. If you want to use stained ZOOM® Strips in 2D electrophoresis, you need to optimize your electrophoresis settings and protocols.

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## Materials Needed

- Sulfosalicylic Acid (SSA) 20% (w/v) solution (Fisher Catalog no. SA168-1) or SSA solid (Fisher Catalog no. BP177)
  - Ultrapure Water
  - Colloidal Blue Staining Kit
  - ZOOM® Equilibration Tray
  - Rotary shaker
- 

## Staining Procedure

Follow the steps below to stain the ZOOM® Strips after IEF:

1. In a clean glass bottle wrapped in aluminum foil, prepare a 1X SSA solution as follows:
    - 3.5% (w/v) Sulfosalicylic Acid
    - 1.0% (v/v) Stainer B from Colloidal Blue Staining KitYou need 15 mL of 1X SSA solution per staining procedure using the ZOOM® Equilibration Tray. You can make a larger volume of SSA for multiple procedures. Store the bottle at room temperature in a fume hood or an acid cabinet.
  2. Remove the ZOOM® IPGRunner™ Cassette from the ZOOM® IPGRunner™ as described in **Disassembling the ZOOM® IPGRunner™**, page 28.
  3. Blot any excess liquid from the cassette using a paper towel. Ensure that your hands are dry.
  4. Firmly hold the ZOOM® IPGRunner™ Cassette and peel off the film cover from the cassette with your dry hands. Avoid introducing any liquid on the cassette surface.

**Note:** You may stain less than 6 strips using the tray. Remove the desired quantity of strips from the cassette as described on page 28 and store the strips in a sealed container at -80°C. When you are ready to stain, place the strips in a cassette and proceed to staining as described below.
  5. Remove one ZOOM® Equilibration Tray from the package and pull on the adhesive liner tab to remove the liner.
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*Continued on next page*

## Staining ZOOM<sup>®</sup> Strips, continued

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### Staining Procedure, continued

6. Hold the ZOOM<sup>®</sup> Equilibration Tray (adhesive side down) and align the tray between the two ribs (protrusions) of the cassette such that the Life Technologies logo is towards the flat end of the cassette. **Place the tray on the cassette and apply firm pressure to the tray on the adhesive area with the blunt end of a pen to ensure a tight seal between the tray and cassette.** A tight seal is formed when the adhesive changes from a cloudy to clear appearance.
  7. Add 10–15 mL of 1X SSA solution through either spout on the tray. If you notice leaking into the adhesive area between the tray and the cassette, check the seal and verify that you are not using more than 15 mL of solution. The ZOOM<sup>®</sup> Strips should be fully submerged in the solution.
  8. Incubate the strips for 1 hour with gentle agitation on a rotary shaker. Bands may appear within 15 minutes. You may analyze bands after 30 minutes, though we recommend incubating for 1 hour. You may incubate the strips for up to 2 hours if necessary.
  9. After staining, decant the remaining solution through the spouts on the tray into a designated waste container. Shake out any residual stain.
  10. Remove the ZOOM<sup>®</sup> Equilibration Tray from the cassette by first pulling on the corners of the tray and then firmly remove the tray. If desired, you may use a gel knife.
  11. Remove the stained ZOOM<sup>®</sup> Strips from the cassette using forceps.
  12. Rinse the strips three times with fresh volumes of ultrapure water. If you do not rinse the strips, the bands will turn yellow and fade. After rinsing, the strips are ready for analysis.
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# Overview of Two-Dimensional Gel Electrophoresis

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## Introduction

Two-dimensional (2D) gel electrophoresis is a powerful and sensitive technique for separating and analyzing protein mixtures from biological samples. 2D gel electrophoresis is performed in two consecutive steps, IEF and SDS-PAGE, as described in the following sections.

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## Sample Preparation

**Sample preparation is a critical step in 2D analysis.** Proper sample preparation is key to the success of a 2D experiment. Many factors play an important role in designing a procedure for sample preparation. See page 6 for **Sample Preparation Guidelines**.

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## 2D Gel Electrophoresis

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 or IPG (immobilized pH gradient) strips (ZOOM® Strips) containing a 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 when they reach their isoelectric point. For more details on IEF, see page 49.
  2. Second dimension separation of proteins using SDS-PAGE.  
Proteins are separated based on their molecular weight using denaturing polyacrylamide gel electrophoresis (SDS-PAGE). For more details on SDS-PAGE, see page 51.
  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. See page 53 for more details on detection methods.
  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.
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## Applications of 2D Gel Electrophoresis

The major applications of 2D gel electrophoresis are:

- Analyzing proteome profiles
  - Detecting post- or cotranslational modifications
  - Discovering new drug targets
  - Studying protein expression in normal, disease, or developmental states
  - Identifying novel proteins
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*Continued on next page*

# Overview of Two-Dimensional Gel Electrophoresis, continued

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## Background Information

### IEF

IEF is an electrophoretic technique for the separation of proteins based on their isoelectric point (pI). The pI of a protein is the pH at which a protein has no net charge and will not migrate in an electric field. The net charge on a protein is due to the presence of its charged side chains, the amino- and carboxyl- termini, and any post-translational modifications. When a protein is placed in a pH gradient and electric current is applied to the system, the protein will migrate in the gradient to a point where the net charge of the protein is zero, or at its pI. To obtain high resolution and the best results, IEF is usually performed under denaturing and reducing conditions and at high voltage. The steps of IEF are described on page 49.

IEF and two-dimensional gel electrophoresis were developed almost 25 years ago. For a review on IEF, see Righetti, 1983. In this method, IEF is performed in tube gels (polyacrylamide gels cast in long glass tubes) using carrier ampholytes. The protein sample is applied to the gel and IEF is performed at high voltage. Once the current is applied, the carrier ampholytes form a continuous pH gradient. While the gradient is formed, the protein migrates in the pH gradient to its pI. At the end of IEF, gel is extruded from the tube and equilibrated in buffer containing SDS to prepare the proteins for migration into the second dimension SDS gel. The tube gel is placed on top of a polyacrylamide gel containing SDS and subjected SDS-PAGE.

### Disadvantages of Carrier Ampholytes

The major disadvantages of performing IEF using carrier ampholytes are:

- pH gradients susceptible to cathodic drift (Righetti, 1983)
- Poor reproducibility of the IEF separation due to batch-to-batch variability of ampholytes
- Altered protein mobility due to binding of carrier ampholytes to the proteins
- Low mechanical strength of the tube gels resulting in gel breakage

### IPG Strips

Using immobilized pH gradient (IPG) gels for first dimension IEF eliminates the problems associated with carrier ampholytes. The IPG technique was developed by Bjellqvist et al and Görg et al (Bjellqvist et al., 1982; Görg et al., 1985). For a review on IPG, see published references (Görg et al., 2000; Righetti, 1990; Righetti & Bossi, 1997). The immobilized pH gradient (IPG) gel is formed by casting polyacrylamide gels using acrylamido buffers (acrylamide derivatives containing charged groups) on a plastic backing. Since the pH gradient is cast into the gel, the gradient is more stable and reproducible. After the IPG gel is polymerized, the gel is washed, dried, and cut into narrow strips (IPG Strips). The pH gradient on the IPG strip can be linear or non-linear (NL). The non-linear gradient is usually expanded from pH 4–7 resulting in a sigmoidal pH gradient. The non-linear IPG strips are useful for analyzing samples containing many proteins with pI in the range of 4–7, which is typical for most crude lysates from all species.

### Advantages of IPG Strips

The following are the advantages of using IPG strips for first dimension IEF:

- More stable and reproducible pH gradients
- Reduced cathodic drift
- Higher mechanical strength of IPG strips because the strips are cast on a plastic backing minimizing gel breakage
- Higher protein loading capacity due to the sample loading method

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*Continued on next page*

# Overview of Two-Dimensional Gel Electrophoresis, continued

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## Critical Steps

The critical steps for the success of a 2D experiment are described below. If the desired results are not obtained after a 2D experiment, it is important to review each of the steps described below to determine a possible cause of failure. See **Troubleshooting** on page 35 for more details.

### Sample preparation

Proper sample preparation is key to the success of a 2D experiment. It is important to have some knowledge of your sample prior to sample preparation. Many components of the sample and sample rehydration buffer or the steps of sample preparation may affect the 2D results.

- High conductive ions (salts) in the sample can cause poor resolution and an increase in the focusing time.
- Sample containing low concentration of denaturants/solubilizers result in precipitation of proteins during rehydration or IEF.
- Preparing samples in the absence of protease inhibitors may result in protein loss due to proteolysis producing smeary or multiple spots on the 2D gel.

Review **Sample Preparation Guidelines** on page 6 for more details.

### pH gradient

It is important to select an IPG strip in the appropriate pH range for your sample to obtain good resolution. If you are unsure of the pH range of your sample, use a broad range strip, such as pH 3–10 IPG strip. If you are interested in resolving proteins only in a narrow pH range, use narrow pH range strips. Many protein samples contain a high percentage of proteins in the pH range of 4–7. Using pH strips with non-linear gradients produce good resolution in these samples. Choosing an incorrect pH range strip for your protein sample will result in protein precipitation or missing spots on the 2D gel.

### Protein load

Loading inappropriate amounts of protein on the IPG strip will result in poor resolution (high load) or no spots (low load). It is very critical to use an accurate and sensitive protein estimation method (see page 11). The protein load will depend on the:

- **Goal of the 2D experiment**  
If the goal is to study the complete protein profile of the sample, load low to medium protein amounts to produce a clean 2D map. If the goal is to study low abundance proteins, load high protein amounts of a prefractionated or enriched sample.
- **pH gradient of the IPG strip**  
Higher protein loads can be loaded on a narrow pH range strip as only a small portion of the total proteins will be resolved on a narrow pH range strip.
- **Detection method**  
The protein spots on the second dimension SDS gel are visualized as spots using a sensitive detection method (see page 53 for more details). Using an improper detection method will result in smearing or missing spots on the 2D gel.

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## Overview of Two-Dimensional Gel Electrophoresis, continued

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### Critical Steps, continued

#### IEF protocol

Using a suitable IEF protocol for your sample will result in sharp, tight spots on the 2D gel. The IEF protocol is dependent on the sample and the IEF apparatus. Follow the manufacturer's recommendations for the IEF protocol to obtain good resolution. Based on your initial results, you may then optimize the focusing conditions for your sample. Samples containing high conductive ions or high protein loads will require longer focusing time.

It is important to have a power supply capable of operating at high voltage and low current. The final high voltage step in IEF (see page 49) is necessary to focus proteins into narrow zones and produce sharp spots on the 2D gel. Inability of the power supply to operate at high voltage and low current may result in less than optimal resolution of proteins.

#### Detection method

The protein spots on the second dimension SDS gel are visualized as spots using a sensitive detection method, such as fluorescent or silver staining (see page 53 for details). Using an improper detection method will result in smearing or missing spots on the 2D gel.

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# First Dimension IEF

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## Introduction

First dimension IEF is performed in two steps:

1. Rehydrating IPG strips.
2. Performing IEF.

Details on each step are described in this section.

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## Rehydrating IPG Strips

IPG strips are supplied dry and have to be rehydrated using an appropriate rehydration buffer. Rehydration allows the gel to swell to its original thickness and is usually performed for 8–12 hours. Because rehydration is performed at room temperature, it is very important that sample proteins remain in solution during the rehydration step. ZOOM® Strips are specifically formulated to allow rehydration in 1 hour.

Rehydration is performed using active or passive methods. ZOOM® Strips are rehydrated in the ZOOM® IPGRunner™ Cassette by passive rehydration.

The rehydration buffer contains urea, non-ionic or zwitterionic detergent, reducing agent, ampholyte solution, and a dye. The function of the rehydration buffer is to completely denature proteins and maintain proteins in solution for isoelectric focusing.

Proteins are loaded on the IPG strips by two methods:

1. In gel rehydration or applying the protein sample during rehydration  
This method of sample application allows you to load dilute protein samples and higher amounts of protein on the IPG strips (Rabilloud et al., 1994).  
The recommended method for sample application on the ZOOM® IPGRunner™ System is in gel rehydration.
  2. Applying the protein sample to the rehydrated IPG strip.
- 

## Performing IEF

IEF is performed using horizontal or vertical IEF systems. The ZOOM® IPGRunner™ System is a unique system providing oil-free first dimension IEF in a vertical mini-gel format. IEF of up to 12 samples is completed in less than 2 hours. Since IEF is performed at high voltage (see **Power Requirements**, next page) it is necessary to have a cooling system or a heat sink built into the IEF system to dissipate heat.

The time required to complete IEF is dependent on many factors and may need optimization based on the initial results. The factors affecting focusing time are:

- Conductive ions (high salt) in the sample
- pH gradient
- High protein load
- Crude lysates

The focusing time is indicated as volt-hours. The total volt-hours will depend on the IEF apparatus and protein sample. In general, a total of 6000–20,000 volt-hours are needed to focus proteins using conventional IEF systems.

The ZOOM® IPGRunner™ is designed for optimal electrical efficiency allowing you to perform quality IEF separation at 2000 volts (1200–1300 volt-hours) unlike traditional IEF systems that operate at high voltage.

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## First Dimension IEF, continued

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### Power Requirements

To obtain the best results, IEF is typically performed by increasing the voltage gradually and maintaining the final focusing voltage for 1–2 hours. It is important to have a power supply capable of operating at high voltage and low current. See page 13 for power supply requirements.

During IEF, proteins migrate in an electric field until the proteins reach their pI and small, charged molecules migrate to the anode/cathode based on the charge of the molecule. At the start of IEF run, the current is high due to the movement of charged molecules. As the proteins start to focus at their respective pI's, the current decreases slowly. Once the proteins have reached their pI, the resulting current is very low (usually  $\mu\text{A}$ ) as there is minimal movement of ions. At that point, a high final voltage is applied to focus the proteins into sharp, narrow zones. Increasing the voltage slowly allows removal of any charged molecules in the protein sample from the strips and prevents the IPG strips from being exposed to very high currents that may damage the protein sample or IEF apparatus.

Alternatively, IEF can be performed at constant wattage so the voltage will increase as the current decreases. However, this requires a longer time.

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# Second Dimension SDS-PAGE

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## Introduction

Second dimension SDS-PAGE is performed in three steps:

1. Equilibrating IPG strips after IEF.
2. Applying IPG strips to the second dimension SDS gel.
3. Performing SDS-PAGE.

Details on each step are provided in the following sections.

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## Equilibrating IPG Strips

Incubating the IPG strips after IEF in an equilibration buffer equilibrates the strips in SDS buffer and prepares the strips for 2D SDS-PAGE. The equilibration buffer usually contains a buffer, reducing agent, SDS, glycerol, iodoacetamide (optional), and dye. Urea in the equilibration buffer is optional. Equilibration is usually performed in two steps:

- **Reduction**

The IPG strip is incubated in a reducing solution for 15 minutes. The reducing solution contains DTT at a final concentration of 50 mM in equilibration buffer. DTT maintains the proteins in a reduced state.

- **Alkylation (optional)**

The IPG strip is incubated in an alkylating solution for 15 minutes. The alkylating solution contains iodoacetamide at a final concentration of 125 mM in equilibration buffer. Iodoacetamide alkylates the sulfhydryl groups on the proteins and DTT. Alkylating the sulfhydryl groups of the proteins reduces vertical streaking of protein bands (Görg et al., 1987).

We recommend using the ZOOM® Equilibration Tray for easy equilibration of strips in the ZOOM® IPGRunner™ Cassette. We also recommend NuPAGE® LDS Sample Buffer (see page 55) as the equilibration buffer for Tris-glycine gels and NuPAGE® Novex® gels.

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## Second Dimension SDS-PAGE, continued

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### Applying IPG Strips to the Second Dimension SDS Gel

The equilibrated IPG strip is applied to the second dimension gel. The IPG strip is placed on top of the vertical SDS gel and sealed onto the gel using an agarose solution. This fixes the IPG strip onto the SDS gel so the strip does not move into the buffer chamber and also makes sure the strip is in contact with the SDS gel.

It is important that air bubbles are not trapped between the strip and gel while sealing the strip with agarose. Bubbles will prevent the migration of proteins into the SDS gel producing blank areas in the SDS gel after detection.

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### SDS Gel

Second dimension SDS-PAGE is usually performed using the Laemmli (Laemmli, 1970) or NuPAGE® buffer systems. The choice of a second dimension gel is dependent on gel percentage (gradient gels or single percentage gels) and size of the gel (mini-gel format or large-format). A mini-gel format is ideal for rapid screening of large number of samples and generates high-resolution and reproducible results (Ducret et al., 2000; Sanchez & Hochstrasser, 1999).

ZOOM® Gels available from Life Technologies are 8 × 8 cm, 1.0 mm thick pre-cast gradient polyacrylamide gels and are ideal for 2D analysis of proteins following IEF of IPG Strips (see page 29). 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.

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### Performing SDS-PAGE

A variety of apparatus are commercially available for performing SDS-PAGE. If you are using a large-format gel, use a suitable apparatus for large-format gels.

XCell *SureLock*® Mini-Cell available from Life Technologies (see page 55) is recommended for performing SDS-PAGE of mini-gels. The XCell *SureLock*® Mini-Cell's unique design allows quick, easy, and leak-free SDS-PAGE of two mini-gels without any clamps or grease.

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# Detection and Analysis

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## Introduction

The proteins on the second dimension SDS gel are visualized as spots using various detection methods. The detection method must be sensitive and should not modify the proteins for any downstream applications such as mass spectrometry or protein sequencing. The choice of detection method depends on the purpose of the gel (qualitative or quantitative), protein load, and downstream application. The various detection methods are described in the following sections.

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## Fluorescent Staining

Fluorescent staining of 2D gels offers high sensitivity (sub-nanogram level), fast staining protocols, and minimal protein-to-protein variability. Fluorescently stained gels can be viewed with a standard UV or blue-light transilluminator or with a laser scanner.

SYPRO<sup>®</sup> Ruby Protein Gel Stain (see page 57) is a highly sensitive, ready-to-use fluorescent stain for proteins in 1D or 2D gels. This fluorescent stain offers several advantages over silver staining, including a simple staining procedure, a linear quantitation range over three orders of magnitude, less protein-to-protein variability, and compatibility with mass spectrometry and microsequencing.

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## Silver Staining

Silver staining is the most common method of protein detection after 2D electrophoresis. Silver staining is 30-fold more sensitive than colloidal Coomassie G-250 staining and detects proteins at sub-nanogram level.

The SilverQuest<sup>™</sup> Silver Staining Kit (see page 57) is recommended for staining proteins if mass spectrometry is desired. This kit is specifically designed to provide sensitive silver staining compatible with mass spectrometry analysis. Most silver staining kits use aldehyde-based sensitizers which modify the lysine residues on the proteins and prevent complete trypsin digestion (Rabilloud, 1990). The SilverQuest<sup>™</sup> Silver Staining Kit does not include an aldehyde-based sensitizer, improving trypsin digestion and extraction of peptides from the gel.

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## Coomassie Staining

Staining proteins with colloidal Coomassie staining provides nanogram-level sensitivity and clear backgrounds, and is five times more sensitive than traditional Coomassie staining techniques.

SimplyBlue<sup>™</sup> SafeStain (see page 57) is a ready-to-use, proprietary Coomassie G-250 stain that is specially formulated for fast, sensitive detection and safe, non-hazardous disposal. Proteins stained using the SimplyBlue<sup>™</sup> SafeStain are compatible with mass spectrometry analysis, as an alcohol/acetic acid fixing step is **NOT** required with this stain preventing any protein modification.

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## Autoradiography

Autoradiography is the most sensitive method of detection. Protein samples are labeled with <sup>35</sup>S, <sup>3</sup>H, or <sup>32</sup>P. After 2D electrophoresis, the gel is treated with an enhancer (optional), dried, and exposed to film.

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## Detection and Analysis, continued

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### **Blotting**

Proteins are transferred to nitrocellulose or PVDF (polyvinylidene fluoride) using western transfer procedure. The proteins on the membrane are subjected to immunodetection or sequencing. Nitrocellulose or PVDF membranes are recommended for immunodetection. PVDF membranes are recommended for sequencing as PVDF membranes are resistant to the solvents used in protein sequencing.

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### **Mass Spectrometry Analysis**

Proteins separated by 2D gel electrophoresis can be further analyzed by mass spectrometry. Following staining, protein spots are cut out of the gel and digested with proteases (such as trypsin) to generate peptide fragments. The peptide fragments are then analyzed using MALDI-TOF (Matrix Assisted Laser Desorption/Ionization-Time Of Flight) MS (Mass Spectrometry), and a peptide mass fingerprint is generated for the protein. This fingerprint may be screened against various databases to identify the protein.

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## Accessory Products

### Additional Products

Many of the items in the ZOOM® IPGRunner™ System, as well as additional reagents for sample fractionation and downstream analysis, are available separately. Ordering information is provided below.

For more information or to order, visit [www.lifetechnologies.com](http://www.lifetechnologies.com) or call Technical Support (page 58).

### Electrophoresis Products

The following electrophoresis products can be used with the ZOOM® IPGRunner™ System.

Product	Quantity	Catalog no.
ZOOM® IPGRunner™ Cassettes	10 each	ZM0003
ZOOM® IPGRunner™ Buffer Core	1 each	ZM0006
ZOOM® IPGRunner™ Lid	1 each	ZM0005
NuPAGE® Novex® 4–12% Bis-Tris ZOOM® Gel	10 gels	NP0330BOX
Novex® 4–20% Tris-Glycine ZOOM® Gel	10 gels	EC60261BOX
XCell SureLock® Mini-Cell	1 kit	EI0001
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
UltraPure™ Agarose	100 g	16500-100
ZOOM® Equilibration Tray	10 trays	ZM0007
NuPAGE® LDS Sample Buffer (4X)	250 mL	NP0008
NuPAGE® Sample Reducing Agent (10X)	10 mL	NP0009
UltraPure™ Dithiothreitol (DTT)	5 g	15508-013
NuPAGE® Antioxidant	15 mL	NP0005
Novex® Power Supply Adapters	1 set	ZA10001
ZOOM® Dual Power Supply (100–120 VAC 47–60 Hz)	1 each	ZP10001
ZOOM® Dual Power Supply (220–240 VAC 47–60 Hz)	1 each	ZP10002

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## Accessory Products, continued

### Sample Preparation Products

The following sample preparation kits can be used with the ZOOM® IPGRunner™ System.

Product	Quantity	Catalog no.
ZOOM® 2D Protein Solubilizer Kit	1 × 5 mL Solubilizer 1 1 × 5 mL Solubilizer 2	ZS10003
ZOOM® 2D Protein Solubilizer 1	4 × 5 mL	ZS10001
ZOOM® 2D Protein Solubilizer 2	4 × 5 mL	ZS10002
Qubit® Protein Assay Kit	1000 assays	Q33210

### Sample Fractionation Products

The following sample fractionation products can be used with the ZOOM® IPGRunner™ System.

Product	Quantity	Catalog no.
ZOOM® IEF Fractionator	1 each	ZF10002
ZOOM® IEF Fractionator Combo Kit	1 kit	ZF10001
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	0.5 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

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## Accessory Products, continued

### ZOOM® Strips and Ampholytes

The following ZOOM® Strips and ZOOM® Carrier Ampholytes can be used with the ZOOM® IPGRunner™ System.

Product	Quantity	Catalog no.
ZOOM® Strip pH 3–10NL (non-linear)	12 strips	ZM0011
ZOOM® Strip pH 4–7	12 strips	ZM0012
ZOOM® Strip pH 6–10	12 strips	ZM0013
ZOOM® Strips pH 4.5–5.5	12 strips	ZM0014
ZOOM® Strips pH 5.3–6.3	12 strips	ZM0015
ZOOM® Strips pH 6.1–7.1	12 strips	ZM0016
ZOOM® Strip pH 3–10L (linear)	12 strips	ZM0018
ZOOM® Strips pH 9–12	12 strips	ZM0017
ZOOM® Carrier Ampholytes pH 3–10	10 mL	ZM0021
ZOOM® Carrier Ampholytes pH 4–7	10 mL	ZM0022
ZOOM® Carrier Ampholytes pH 6–9	10 mL	ZM0023
ZOOM® Carrier Ampholytes pH 9–11	10 mL	ZM0024
ZOOM® Carrier Ampholytes pH 4–6	10 mL	ZM0025

### Protein Detection Products

The following stains and markers can be used with the ZOOM® IPGRunner™ System.

Product	Quantity	Catalog no.
SYPRO® Ruby Protein Gel Stain	200 mL	S12001
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	LC5602

# Technical Support

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**Obtaining Support** For the latest services and support information for all locations, go to [www.lifetechnologies.com](http://www.lifetechnologies.com)

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