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Instruction Manual

E-PAGE™ *96* Protein Electrophoresis System

For high-throughput electrophoresis of proteins

Catalog nos. EP096-06, EPST96-06

Version C
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E-PAGE™ Experienced Users Procedure

Introduction

This quick reference sheet is included for experienced users of E-PAGE™ *96* Gels. If you are a first time user, follow the detailed protocols provided in this manual.

Note: For optimal results, load each E-PAGE™ *96* Gel within 30 minutes of removing the gel from the plastic pouch and run within 15 minutes of loading.

Step	Action
Prepare Sample	Use up to 20 µg protein per lane of the E-PAGE™ *96* Gel. See page 4 for sample preparation.
Align Robotic Tip Assembly	If you are using automated loading, align the robotic tip assembly as described below. 1. Set position of the first tip approximately 1 mm above the slope of the A1 well (Figure 2, page 10) to ensure that the remaining tips are aligned above the slopes of the other wells. 2. Refer to the manufacturer's manual for your automated liquid handling system to program this setting. Proceed to loading the gel.
Select Program and Load	1. Plug the Mother E-Base™ into an electrical outlet. 2. If using a Daughter E-Base™, connect the Daughter E-Base™ to a Mother E-Base™ or another Daughter E-Base™ connected to a Mother E-Base™. 3. Press and release pwr/prg (power/program) button to select the **program EP** for E-PAGE™ *96* Gels. 4. Remove gel from the package and remove the plastic comb from the gel. 5. Slide the gel into the two electrode connections on the Mother E-Base™ or Daughter E-Base™. 6. Load samples into the gels using a multichannel pipetter or an automated liquid handling system. First load deionized water into each well and then load your samples as described below: **First Load Deionized Water** — **Then Load Sample in Loading Buffer or MW marker** 20 µl — 5-10 µl 19-10 µl — 11-20 µl 7. Load the appropriate protein molecular weight marker in the marker wells of the gel.

Continued on next page

E-PAGE™ Experienced Users Procedure, Continued

Step	Action
Electrophoresis with E-Base™	1. Press and release the pwr/prg button located on the lower right corner of the base to begin electrophoresis. 2. The Mother E-Base™ and Daughter E-Base™ will signal the end of the run with a **flashing red** light and **rapid beeping for 2 minutes followed by a single beep every minute**. 3. Press and release the pwr/prg button to stop the beeping. 4. Remove the E-PAGE™ *96* cassette from the base. 5. Open the gel cassette for staining or blotting applications.
Opening the Cassette	Open the E-PAGE™ *96* cassette with the Butterfly Opener (included in the kit) and remove the gel. 1. Insert the wide side of the red Butterfly Opener between the tabs at the edge of the E-PAGE™ *96* cassette and twist to separate the two halves of the cassette. 2. Pull apart the cassette halves with your hands until the cassette halves are separated. 3. Using the Butterfly Opener or a gel knife, trim the top and bottom electrode areas of the gel. 4. Proceed to staining or blotting.
Staining and Blotting	• For Coomassie® R-250 staining, see page 18. • For semi-dry blotting, see page 21. E-PAGE™ *96* gels may be stained using many protein staining methods. For details on other staining and blotting methods, refer to the E-PAGE™ Technical Guide at www.invitrogen.com or by contacting Technical Service (page 34).
Using E-Editor™ 2.02 Software	1. Use an appropriate digital documentation system to capture a digital image of the gel. 2. Download E-Editor™ 2.02 software and the instruction manual for free at www.invitrogen.com/epage. 3. Use the E-Editor™ 2.02 software to align and arrange the lanes in the image and save the reconfigured image for further analysis.

Contents and Storage

Types of Products

This manual is supplied with the following products:

Product	Quantity	Catalog no.
E-PAGE™ *96* 6% Gels	8-pack	EP096-06
E-PAGE™ Starter Kit	1 kit	EPST96-06

Contents

The contents for E-PAGE™ *96* Gels and E-PAGE™ Starter Kit are listed below:

E-PAGE™ *96* Gels	**Quantity**
E-PAGE™ *96* 6% Gels	8 gels
E-PAGE™ Loading Buffer 1 (4X)	4.5 ml
Butterfly Opener	1
E-PAGE™ Blotting Pad	1

E-PAGE™ *96* Starter Kit	**Quantity**
E-PAGE™ *96* 6% Gels	4 gels
E-PAGE™ Loading Buffer 1 (4X)	4.5 ml
Butterfly Opener	1
E-PAGE™ Blotting Pad	1
Mother E-Base™	1
E-PAGE™ SeeBlue® Pre-stained Protein Standard	500 µl

Shipping and Storage

The E-PAGE™ *96* Gels, Loading Buffer 1 (4X), E-PAGE™ Blotting Pad and Mother E-Base™ are shipped at room temperature. E-PAGE™ SeeBlue® Pre-stained Protein Standard is shipped on blue ice.

Upon receipt, store the contents as described below:

- Store E-PAGE™ *96* Gels, E-PAGE™ Blotting Pad and Mother E-Base™ at room temperature. Do not allow the temperature to drop below 4°C or rise above 40°C when storing the gels.
- Store E-PAGE™ Loading Buffer 1 (4X) at room temperature or at 4°C.
- Store E-PAGE™ SeeBlue® Pre-stained Protein Standard at 4°C.

Specifications

Specifications

Each E-PAGE™ *96* Gel contains 96 sample wells and 8 marker wells (M).

Cassette Size:	13.5 cm (l) × 10.8 cm (w) × 0.67 cm (thick)
Gel Formulation:	Proprietary, operating at neutral pH
Separation Range	10-300 kDa
Gel Thickness:	3.7 mm
Gel Volume:	50 ml
Well Depth:	3 mm
Well Opening:	3.8 mm x 1.8 mm
Well Bottom:	3.3 mm × 1.1 mm
Running Distance: (one well to the next)	16 mm
Space between Wells:	9 mm

The well openings of the E-PAGE™ *96* cassette are compatible with a multichannel pipetter or 8-, 12-, or 96-tip automated liquid handling devices.

Product Qualification

E-PAGE™ *96* pre-cast Gels are qualified by running E-PAGE™ SeeBlue® Pre-Stained Protein Standards and BSA under standard running conditions as described in this manual. Gels are visualized for proper resolution and migration of bands. Visual inspection is also performed to ensure that the gels are free from bubbles, spots, and any gel residues.

The E-PAGE™ SeeBlue® Pre-Stained Standard must show 5 distinct bands when separated by electrophoresis on an E-PAGE™ *96* 6% Gel.

Introduction

Overview

Introduction

The E-PAGE™ High-Throughput (HTP) Protein Electrophoresis System is designed for fast, high-throughput protein electrophoresis in a horizontal format.

The E-PAGE™ *96* System consists of the following components:

- E-PAGE™ *96* Pre-cast Gels
- E-Base™ Electrophoresis Device
- E-PAGE™ Loading Buffer
- E-Holder™ Platform
- E-Editor™ 2.02 Software

Applications

The E-PAGE™ HTP Protein Electrophoresis System is ideal for screening protein samples using these applications:

- In-gel staining with Lumio™ Green Reagent
- Staining (Coomassie®, silver, or fluorescent stains)
- Western blotting
- Functional assays

E-PAGE™ *96* Pre-cast Gels

E-PAGE™ *96* Gels are self-contained, pre-cast gels that include a buffered gel matrix and electrodes packaged inside a disposable, UV-transparent cassette.

Each E-PAGE™ *96* Gel contains 96 sample lanes and 8 marker lanes in a patented staggered well format that is compatible for loading with a multichannel pipetter or with an automated liquid handling system in the standard 96-well plate format (see page 10 for specifications).

After electrophoresis, the E-PAGE™ cassette is easily opened with the included Butterfly Opener to remove the gel for staining or blotting applications.

In addition, each E-PAGE™ *96* cassette is labeled with an individual barcode to facilitate identification of the gel using commercial barcode readers (page 9).

Continued on next page

Overview, Continued

Diagram of an E-PAGE™ *96* Cassette

A diagram of the E-PAGE™ *96* gel cassette is shown below.

E-Base™

E-PAGE™ *96* Gels are used with specially designed electrophoresis device that combines a base and a power supply. Two types of devices are available from Invitrogen:

- The **Mother E-Base™** (catalog no. EB-M03) has an electrical plug that can be connected directly to an electrical outlet and is used for electrophoresis of one E-PAGE™ *96* Gel. The Mother E-Base™ has been tested for electrophoresis with up to three Daughter E-Bases™ connected at one time.
- The **Daughter E-Base™** (catalog no. EB-D03) connects to the Mother E-Base™, and together they can be used for the independent electrophoresis of 2 or more E-PAGE™ *96* Gels. The Daughter E-Base™ does not have an electrical plug and cannot be used without a Mother E-Base™.

Refer to page 13 for a diagram of the bases.

Important

The E-PAGE™ *96* Gel is **not compatible** with the E-Gel® *96* bases (cat nos. G7100-01/G7200-01) previously available from Invitrogen. The older E-Gel® *96* bases do not have the 'E-Base™' inscription on the platform.

Continued on next page

Overview, Continued

Loading Buffer

E-PAGE™ *96* Gels are supplied with E-PAGE™ Loading Buffer 1 (4X), which is optimized for the E-PAGE™ System. E-PAGE™ Loading Buffer 1 (4X) is recommended for routine SDS-PAGE and staining or blotting applications. **Do not use any other sample buffer.**

E-Holder™ Platform

The E-Holder™ Platform is designed to hold E-PAGE™ gels during loading. Use the E-Holder™ when you need to load multiple gels while other gels are running on the E-Base™.

Note: The E-Holder™ is not a power supply unit, cannot be connected to an electrical outlet, and cannot be used to run gels.

See page 24 for more information about the E-Holder™ Platform.

E-Editor™ 2.02 Software

The E-Editor™ 2.02 Software allows you to quickly reconfigure digital images of E-PAGE™ *96* results for analysis and documentation (page 29).

E-Editor™ 2.02 software can be downloaded **FREE** at www.invitrogen.com/epage. Follow the instructions to download the software and user manual.

Methods

Sample Preparation

Prepare your protein samples as described in this section for electrophoresis on E-PAGE™ *96* Gels.

We recommend that you read this section before preparing your samples. Based on your method of detection, choose a sample preparation method using the appropriate loading buffer:

Application	Method	Loading Buffer
Routine staining and western blotting	1 (page 7)	Loading Buffer 1
Lumio™ Green Detection	2 (page 8)	Lumio™ Gel Sample Buffer

Note

The E-PAGE™ *96* Gels contain SDS and are designed for performing electrophoresis under denaturing conditions.

To obtain the best results, we recommend performing SDS-PAGE under reducing conditions. If you need to perform SDS-PAGE under non-reducing conditions, omit adding NuPAGE® Sample Reducing Agent (10X) during sample preparation.

Materials Needed

- Protein sample
- NuPAGE® Sample Reducing Agent (10X)
- E-PAGE™ Loading Buffer 1 (4X) included in the kit
- Deionized water
- Heating block set at 70°C
- Molecular weight markers
- **Optional:** Lumio™ Green Detection Kit (for detection of Lumio™ fusion proteins)

See page 33 for ordering information.

Continued on next page

Sample Preparation, Continued

Amount of Protein

Use up to 20 µg protein per well of the E-PAGE™ *96* Gel. The amount of protein required will depend on the staining or western detection method used for visualizing proteins after electrophoresis. If you are unsure of how much to use, test a range of concentrations to determine the optimal concentration for your sample.

The maximum recommended loading amount for E-PAGE™ *96* Gel is 20 µg protein per well. Excess proteins will cause poor resolution.

Note: To ensure a proper LDS (lithium dodecyl sulfate from Loading Buffer 1) to protein ratio, limit sample protein or lipid (from the sample) amount to 2 µg/µl of the final sample volume.

Total Sample Volume

The recommended total sample volume for E-PAGE™ *96* Gels is 10 µl. If desired, you may load between 5-20 µl of sample. For best results, avoid loading less than 5 µl of sample and more than 20 µl of sample.

Prior to sample loading, load 10-20 µl deionized water first into all wells.

High Salt or Detergent Samples

Samples containing high salt or detergents will cause loss of resolution on E-PAGE™ *96* Gels. Dilute the samples such that the final concentration of the salt or detergent in the sample is as described below:

Salt/Detergent	Final Concentration
Triton® X-100	< 0.5%
Tween® 20	<0.5%
SDS	<4%
Tris	<200 mM
NaCl	<250 mM

Continued on next page

Sample Preparation, Continued

Loading Buffer

Use the appropriate loading buffer, based on the application as described below.

- **SDS-PAGE and Routine Staining (Method 1, page 7)**

  For SDS-PAGE and staining or blotting, we recommend using the E-PAGE™ Loading Buffer 1 (4X) (included in the kit) for preparing samples. The E-PAGE™ Loading Buffer 1 is optimized for E-PAGE™ *96* Gels. **Do not use any other SDS-PAGE sample buffer.**

- **SDS-PAGE and In-Gel Detection of Lumio™ Fusion Proteins (Method 2, page 8)**

  For detection of Lumio™ fusion proteins with the Lumio™ Green Detection Kit, use the Lumio™ Gel Sample Buffer (4X) supplied with the Lumio™ Green Detection Kit. This buffer is specifically formulated to provide optimal results with the Lumio™ Green Detection Reagent. **Do not use E-PAGE™ Loading Buffer 1 to prepare samples for Lumio™ Green Detection.**

Continued on next page

Sample Preparation, Continued

Molecular Weight Standards

To obtain the best results, we recommend using the following protein molecular weight standards. See page 25 for molecular weight standard calibration in E-PAGE™ *96* Gels. See page 33 for ordering information.

Marker	Amount	Application
E-PAGE™ SeeBlue® Pre-stained Protein Standard	10 µl	Electrophoresis
E-PAGE™ MagicMark™ Unstained Protein Standard	5 µl	Western Blotting
BenchMark™ Fluorescent Protein Standard	5 µl	Lumio™ Detection

Method 1: Routine Staining and Blotting

Use this protocol if you are performing SDS-PAGE followed by routine staining or blotting.

If the E-PAGE™ Loading Buffer 1 (4X) is stored at 4°C, bring the buffer to room temperature and mix briefly prior to use.

1. Prepare your samples in a total volume of 10 µl in E-PAGE™ Loading Buffer 1 as described below. If you need to prepare samples in a volume of 5-20 µl, adjust the volume accordingly.

Reagent	Reduced	Non-Reduced
Protein Sample	x µl	x µl
E-PAGE™ Loading Buffer 1 (4X)	2.5 µl	2.5 µl
NuPAGE® Sample Reducing Agent (10X)	1 µl	--
Deionized Water	to 10 µl	to 10 µl

2. Incubate the samples at 70°C for 10 minutes.
3. Proceed to **Loading E-PAGE™ *96* Gels**, page 9.

Continued on next page

Method 2: Lumio™ Detection

Use this protocol to prepare samples for specific detection of Lumio™ fusion proteins with the Lumio™ Green Detection Kit.

For details on Lumio™ Green Detection Kit, refer to the product manual at www.invitrogen.com or contact Technical Service (page 34).

1. Refer to the Lumio™ Detection manual for details on each type of protein. Prepare protein samples as follows:

Protein Sample	Sample Volume	Lumio™ Gel Sample Buffer (4X) Volume
Bacterial samples	15 µl	5 µl
Mammalian lysate	15 µl	5 µl
Partially purified sample	15 µl	5 µl
Purified sample	15 µl	5 µl
In vitro expressed	20 µl	Not needed*

   *There is no need to add Lumio™ Gel Sample Buffer (4X) as the sample is already prepared in this buffer.

2. Thaw the Lumio™ Green Detection Reagent and mix well.
3. To the protein samples from Step 1, add 0.2 µl Lumio™ Green Detection Reagent in a fume hood. Mix well. Return the Lumio™ Green Detection Reagent to -20°C immediately after use.
4. Incubate samples at 70°C for 10 minutes.
5. Allow samples to cool for 1-2 minutes and centrifuge briefly at maximum speed in a microcentrifuge.
6. Thaw the Lumio™ In-Gel Detection Enhancer and mix well. Add 2 µl Lumio™ In-Gel Detection Enhancer to the samples.
7. Mix well and incubate the samples at room temperature for 5 minutes. Return the Lumio™ In-Gel Detection Enhancer to -20°C immediately after use.
8. Proceed to **Loading E-PAGE™ *96* Gels** (page 9)**.**

   Note: For electrophoresis of Lumio™ fusion proteins, we recommend running the gel for an additional 2 minutes to prevent the formation of a fluorescent dye front.
9. After electrophoresis is complete, immediately visualize and image the gel as described on page 16.

Loading E-PAGE™ *96* Gels

Introduction

After preparing your samples, you are ready to load the E-PAGE™ *96* Gel. This section describes the procedure for loading protein samples and molecular weight standards on E-PAGE™ *96* Gels.

The Mother E-Base™ and Daughter E-Base™ are designed to fit most robotic platforms allowing you to load and run E-PAGE™ *96* Gels directly on an automated liquid handling system.

If you need to load multiple gels on a robotic platform while other gels are running on the E-Base™, use an E-Holder™ Platform (page 24).

If you are using an automated liquid handling device, it is important to align the robotic tip loading assembly to the proper setting prior to loading samples on the E-PAGE™ *96* Gel. This ensures proper loading of samples into the wells. See next page for more details.

- Dispose of gels as hazardous waste.
- Avoid touching the gel while the gel is running.

Using the Barcode

Each E-PAGE™ *96* Gel is labeled with an individual barcode. The barcode facilitates the identification of each gel cassette during electrophoresis of multiple gels. Each E-PAGE™ *96* Gel contains an EAN 39 type of barcode, which is recognized by the majority of commercially available barcode readers. Refer to the manufacturer's instructions to set up the barcode reader.

Note: When capturing an image of the E-PAGE™ *96* Gel, note that the barcode label is easily overexposed. To ensure that the barcode label is distinct and readable in the image, experiment with different shutter settings for your particular camera.

Continued on next page

Loading E-PAGE™ Gels, Continued

Aligning the Robotic Loading Assembly

The wells of the E-PAGE™ *96* Gel are staggered to provide maximum run length (Figure 1, below). For proper sample loading, it is important to program your automated liquid handling system to set the A1 tip of the 8-, 12-, or 96-tip robotic head over the E-PAGE™ *96* Gel as described below.

Set the position of the first tip approximately 1 mm above the slope of the A1 well (Figure 2, below). This will ensure that the remaining tips are aligned above the slopes of the remaining wells. Refer to the manufacturer's manual of your automated liquid handling system to program this setting. After programming the setting, load your samples. During loading, the samples will fall onto the slopes of the wells and be drawn into the wells by capillary force.

Figure 1

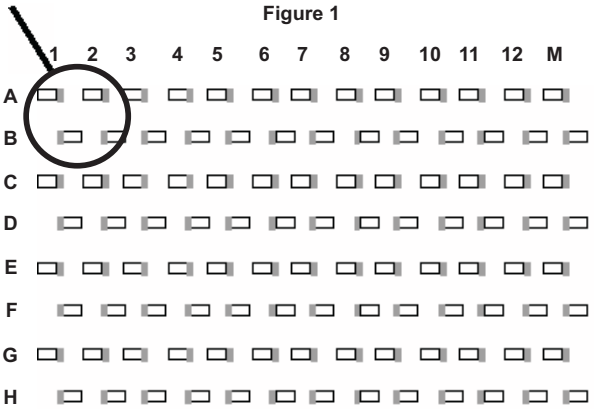
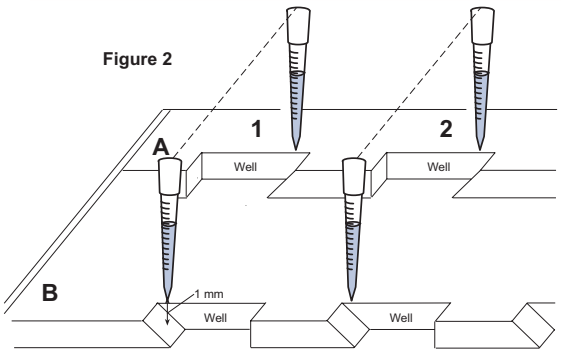


Figure 2



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Loading E-PAGE™ *96* Gels, Continued

Selecting a Program on E-Base™

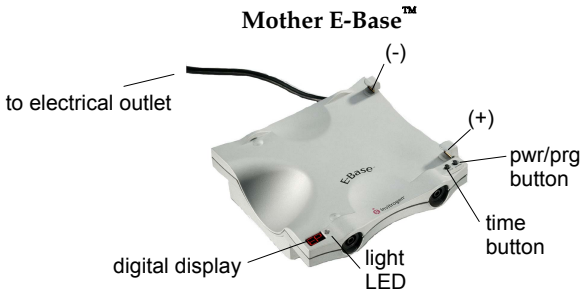
The recommended program for electrophoresis of the E-PAGE™ *96* Gel is program EP, which runs for 14 minutes.

Brief instructions for using the E-Base™ are included in the following sections. For further details, refer to the E-Base™ manual, available at www.invitrogen.com.

You need to select program EP on the base prior to inserting a gel.

1. Plug the Mother E-Base™ into an electrical outlet using the electrical plug on the base.

   If using Daughter E-Base™, connect the Daughter E-Base™ to a Mother E-Base™ or another Daughter E-Base™ connected to a Mother E-Base™.
2. The digital display shows EP or last program used.
3. Press and release the pwr/prg (power/program) button to select the **program EP** for E-PAGE™ *96* Gels. The digital display shows EP.



Important

- To obtain the best results, run the E-PAGE™ *96* Gel immediately after removal from the pouch and loading.
- Store and run E-PAGE™ *96* Gels at room temperature.
- **Always load 10-20 µl deionized water first into all wells prior to sample loading.**
- For optimal results, we do not recommend running reduced and non-reduced samples on the same gel. If you do choose to run these samples on the same gel, avoid running reduced and non-reduced samples in adjacent lanes as the reducing agent may have a carry-over effect on the non-reduced samples.
- Avoid running samples containing different salt or protein concentrations in adjacent lanes.

Continued on next page

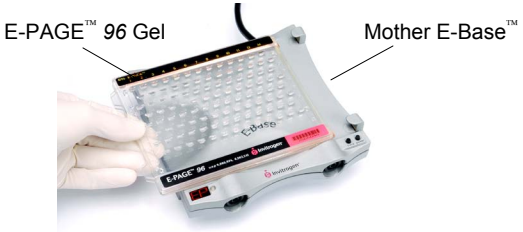
Loading E-PAGE™ *96* Gels, Continued

Loading E-PAGE™ Gels

Each E-PAGE™ *96* Gel is supplied individually wrapped and ready for use.

Use short, rigid tips for loading.

1. Open the package and remove the E-PAGE™ *96* Gel.
2. Remove the plastic comb from the gel.
3. Slide the gel into the two electrode connections on the Mother or Daughter E-Base™. The two copper electrodes on the right side of the cassette must be in contact with the two electrode connections on the base.



When the gel is properly inserted into the base, a fan in the base begins to run, a red light illuminates at the lower left corner of the base, and the digital display shows 14 minutes for program EP or last time setting.

Note: If you accidentally inserted a cassette into the base before selecting program EP, remove the cassette, select program EP, and reinsert the cassette. To add to the run time of 14 minutes, press the time button until the desired time is displayed and then release the time button.

4. Load deionized water to each well of the E-PAGE™ *96* Gel prior to loading your samples or protein molecular weight standards. Load samples into the gels using a multichannel pipetter or a liquid handling system as described below:

First Load Deionized Water	Then Load Sample in Loading Buffer
20 µl	5-10 µl
19-10 µl	11-20 µl

5. Load the appropriate protein molecular weight marker in the marker wells of the gel. See page 7 for recommended molecular weight standards.
6. Proceed immediately to **Electrophoresis**, next page.

Electrophoresis of E-PAGE™ *96* Gels

Introduction

After loading your protein samples on the E-PAGE™ *96* Gels, proceed immediately to electrophoresis using the E-Base™. See page 26 for electrophoresis results of E-PAGE™ *96* Gels.

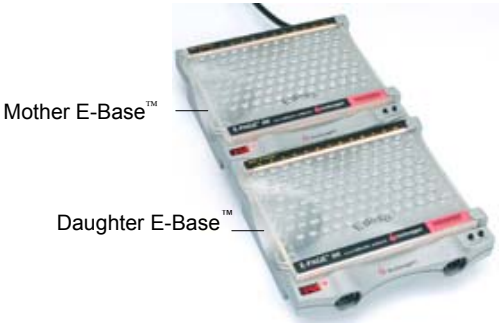
Using an E-Base™

Instructions for running an E-PAGE™ *96* Gel in a Mother E-Base™ or Daughter E-Base™ are provided below. For more details on setting the time or interrupting a run, refer to the manual supplied with the E-Base™.

Note: It is not necessary to have a gel in the Mother E-Base™ if you are using a Daughter E-Base™. However, the Mother E-Base™ must be plugged into an electrical outlet.

1. To begin electrophoresis, press and release the pwr/prg (power/program) button located on the lower right corner of the Mother E-Base™.

   If you are using a Daughter E-Base™, press and release the pwr/prg button located on the lower right corner of the Daughter E-Base™.



The **red light** will change to a **green light** and the digital display will show the count down time during the run.

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Electrophoresis of E-PAGE™ *96* Gels,
Continued

Using an E-Base™, continued

While the run is in progress, you can add to the run time by pressing the time button to select the desired time and then release the time button. The recommended running time for an E-PAGE™ *96* Gel is 14 minutes. **Avoid running an E-PAGE™ *96* Gel for more than 25 minutes.**

To interrupt or stop a run in progress, see next page.

Note: If your sample contains high salt or detergent concentrations, you may need to manually increase the run time. For electrophoresis of Lumio™ fusion proteins, we recommend running the gel for an additional 2 minutes to prevent the formation of a fluorescent dye front.

2. The Mother E-Base™ and Daughter E-Base™ signals the end of the run with a **flashing red light** and rapid beeping for 2 minutes followed by a **single beep** every minute.

   At the end of the run, the digital display shows the original time setting (not any time change that was made during the electrophoresis). The digital display also shows the elapsed time (up to 19 minutes with a negative sign) since the end of the run.

3. Press and release the pwr/prg button to stop the beeping. The light turns to a **steady red** and the digital display shows the last time setting.

4. Remove the gel cassette from the Mother E-Base™ and Daughter E-Base™. **Note: The bands in the gel will diffuse within 40 minutes.**

5. For staining or blotting the gel, see pages 18 - 23. For visualizing Lumio™ fusion proteins, see page 16.

Continued on next page

Electrophoresis of E-PAGE™ *96* Gels,
Continued

Interrupting a Run

You can interrupt an electrophoresis run at any time by **pressing and releasing** the pwr/prg button to stop the current. The stopped current is indicated by a **steady red light**, and the digital display will flash to indicate that the run has been interrupted.

You can remove the gel from the base to check the progress of the run. Then:

- To **continue** the run from the point at which it was stopped, reinsert the gel and press and release the pwr/prg button. The light changes to steady green and the digital display shows the count down time.
- To **cancel** the rest of the interrupted run, press and hold the pwr/prg button for a few seconds. The digital display will reset and the base will return to Ready Mode. If desired, you can then program a new run time and rerun the gel.

In case of an **external power failure**, the run will continue when the power resumes. The Mother E-Base™ and Daughter E-Base™ will signal the end of the run as described on the previous page, except the light will be an alternating red/green to indicate that an external power failure has occurred during the run.

Note

We recommend that you disconnect the Mother E-Base™ from the outlet when not in use for a prolonged period of time.

Maintaining E-Base™

Keep the surfaces of the Mother E-Base™ and Daughter E-Base™ free of contaminants. To clean, disconnect bases from power source and wipe with a dry cloth. Do not attempt to open or service the bases. To honor the warranty, bases should only be opened and serviced by Invitrogen.

Visualizing Lumio™ Fusion Proteins

Introduction

The steps involved in detecting Lumio™ fusion proteins run on an E-PAGE™ *96* Gel are described below.

To visualize Lumio™ fusion protein bands in the gel after electrophoresis, you will need a UV transilluminator or a laser-based scanner (see below). For further details on imaging Lumio™ fusion proteins, refer to the Lumio™ Green detection kit manual available at www.invitrogen.com or by contacting Technical Services (page 34).

Visualizing Lumio™ Fusion Proteins

After electrophoresis is complete, immediately visualize and image the gel as described below. **There is no need to remove the E-PAGE™ *96* Gel from the cassette to visualize Lumio™ fusion proteins.**

1. Place the cassette on a UV transilluminator equipped with a camera and select the ethidium bromide or SYBR® Green filter on the camera.

   You may also use a laser-based scanner with a laser line that falls within the excitation maxima of the stain (500 nm) and a 535 nm long pass filter or a band pass filter centered near the emission maxima of 535 nm.

   Note: Adjust the settings on the camera prior to turning on the UV transilluminator. Avoid exposing the gel to UV light for long periods of time.

2. Image the gel with a suitable camera with the appropriate filters using a 4-10 second exposure. You may need to adjust the brightness and contrast to reduce any faint non-specific bands.

You should see fluorescent bands of Lumio™ fusion proteins and the gel should have minimum background, as shown on page 28

Opening the E-PAGE™ *96* Cassette

Introduction

Prior to staining or blotting the E-PAGE™ *96* Gel, you need to open the cassette using the red plastic Butterfly Opener and remove the gel.

Opening the Cassette

1. Insert the wide side of the red Butterfly Opener (included in the kit) between the tabs at the edge of the E-PAGE™ *96* cassette and twist to separate the two halves of the cassette (Figure A, below).
2. Gently pull apart the cassette halves with your hands until the cassette halves are completely separated and the gel is exposed (Figure B, below).

A **B**

3. Carefully remove the gel from the cassette.
4. Using the Butterfly Opener or a gel knife, trim the top and bottom electrode areas of the gel.
5. Proceed to staining (next page) or blotting (page 21).

Note

Small pieces of gel material may remain in the wells of an E-PAGE™ Gel after removal of the gel from the cassette. To obtain the best staining and blotting results, remove any small pieces of gel material in the wells of the gel by gently rubbing a gloved hand over the well side of the gel.

Coomassie® R-250 Staining Protocol

Introduction

The instructions for visualizing protein bands on E-PAGE™ *96* Gels with Coomassie® R-250 stain using a microwave protocol are described in this section. Using the microwave protocol reduces the amount of time needed for staining and destaining.

Additional Staining Protocols

You may stain E-PAGE™ *96* Gels using many protein staining methods. For additional staining protocols, refer to the E-PAGE™ Technical Guide available at www.invitrogen.com or by contacting Technical Service (page 34).

Materials Needed

You will need the following items for staining one E-PAGE™ *96* Gel:

- Coomassie® R-250 stain (0.015% in 30% ethanol and 10% acetic acid). See note below.
- Destaining solution (8% acetic acid in deionized water)
- Clean, **microwave safe** staining containers. Do NOT use Incubation Tray (Cat. no. LC2102)
- 2 pieces of nylon membrane (Cat. no. LC2003)
- Rotary shaker
- Microwave oven

Note

The volume of staining and destaining solutions needed will depend on the volume of the staining container. The volume of all solutions must be sufficient to cover the gel completely.

Important

When using the microwave staining protocol, warm the staining and destaining solutions to 50°C without boiling. **It is important NOT to boil the solutions**.

Since microwave ovens differ significantly, we recommend testing various times (at 10 second intervals) and power settings of your microwave oven to achieve a temperature of 50°C in the volume of solution required for your particular staining container. Perform these steps without the gel. Once you have optimized the time and settings for your microwave oven, use these settings for staining.

Continued on next page

Coomassie® R-250 Staining Protocol, Continued

Microwave Staining Protocol

For all staining and destaining steps described below, be sure to use sufficient reagents to completely cover the gel in a **microwave safe** container such that the gel moves freely during the staining and destaining steps.

1. After electrophoresis, remove the gel from the cassette (page 17) and place the gel in a clean **microwave safe** container.
2. Prepare Coomassie® stain (0.015% Coomassie® R-250 in 30% ethanol and 10% acetic acid). See previous page.
3. Add enough stain to completely cover the gel in the **microwave safe** container.
4. Warm the gel and solution to about 50°C in a microwave oven (see previous page). **Note:** Do NOT boil the solution.
5. Incubate the gel in the warmed staining solution for 30 minutes on an orbital shaker at room temperature.
6. Discard the stain, rinse the gel briefly with water and discard the water.
7. Add enough destaining solution (see previous page) to cover the gel during incubation.
8. Place two pieces of positively charged nylon membrane on top of the destaining solution to speed up the destaining process.
9. Warm the destaining solution, gel and nylon membrane to 50°C in a microwave oven (see previous page). **Note:** Do NOT boil the solution.
10. Incubate the gel in the warm destaining solution on an orbital shaker at room temperature until the desired background is achieved.

Note: To obtain a clear background, perform destaining overnight.

Drying E-PAGE™ *96* Gels

Drying the Gel

We recommend using the Large Gel Drying Kit available from Invitrogen to air-dry the gel. Refer to the Large Drying Kit manual or to the E-PAGE™ Technical Guide at www.invitrogen.com or by contacting Technical Service (page 34).

Note: The E-PAGE™ *96* Gel will need at least 4-5 days for complete drying.

Due to the thickness of the E-PAGE™ *96* Gel, vacuum drying is not recommended and may cause the gel to crack.

Semi-Dry Blotting of E-PAGE™ *96* Gels

Introduction

A semi-dry blotting procedure for blotting E-PAGE™ Gels is described in this section. You will need a semi-dry transfer apparatus that is capable of accommodating the dimensions of an E-PAGE™ Gel (8.6 cm x 13.5 cm) and a power supply. For details on semi-wet blotting, refer to the E-PAGE™ Technical Guide available at www.invitrogen.com or by contacting Technical Service (page 34). See page 27 for results obtained with semi-dry blotting of E-PAGE™ *96* Gels.

Materials Needed

You will need the following items. See page 33 for ordering information.

- Semi-dry blotter
- NuPAGE® Transfer Buffer (20X)
- NuPAGE® Antioxidant
- Blotting membranes: Invitrolon™/Filter Paper Sandwiches or Nitrocellulose Membrane/Filter Paper Sandwiches
- E-PAGE™ Blotting Pad (supplied with E-PAGE™ Gels or available separately)
- 4 pieces of 2.5 mm Blotting Filter Paper
- Blotting Roller
- Incubation Tray

2X NuPAGE® Transfer Buffer

We recommend using 2X NuPAGE® Transfer Buffer with NuPAGE® Antioxidant **without methanol** for the optimal transfer of most proteins from E-PAGE™ *96* Gels.

For one gel, prepare 500 ml of 2X NuPAGE® Transfer Buffer as follows:

Buffer Component	2X NuPAGE® Transfer Buffer
NuPAGE® Transfer Buffer (20X)	50 ml
NuPAGE® Antioxidant	0.5 ml
Deionized Water	to 500 ml

Continued on next page

Semi-Dry Blotting of E-PAGE™ *96* Gels,
Continued

Equilibrating the Gel

Equilibration of the gel in 2X Transfer Buffer results in removal of salts that may increase conductivity and heat during transfer. Perform equilibration for the recommended time, as longer equilibration will result in protein diffusion.

1. After electrophoresis, remove the gel from the cassette as described on page 17.
2. Using the Butterfly Opener or a gel knife, trim off the top and bottom electrode areas of the gel.
3. Equilibrate the E-PAGE™ Gel in 200 ml 2X NuPAGE® Transfer Buffer (see previous page) for 30 minutes. Perform this equilibration on a shaker.

Preparing Blotting Membrane

Nitrocellulose

1. Use pre-cut Nitrocellulose/Filter Paper Sandwich or cut nitrocellulose membrane to the appropriate size (8.6 cm x 13.5 cm).
2. Soak the membrane in 2X NuPAGE® Transfer Buffer (see previous page) for several minutes in the Incubation Tray.

PVDF

1. Use pre-cut Invitrolon™/Filter Paper Sandwich or cut PVDF membrane to the appropriate size (8.6 cm x 13.5 cm).
2. Pre-wet the membrane for 30 seconds in methanol, ethanol, or isopropanol. Briefly rinse in deionized water.
3. Soak the membrane in 2X NuPAGE® Transfer Buffer (see previous page) for several minutes in the Incubation Tray

Continued on next page

Semi-Dry Blotting of E-PAGE™ *96* Gels,
Continued

Semi-Dry Blotting Protocol

1. In a clean container or Incubation Tray, soak 4 pieces of 2.5 mm Blotting Filter Paper (8.6 cm x 13.5 cm) in 2X NuPAGE® Transfer Buffer (page 21). Remove any air bubbles trapped between sheets using the Blotting Roller while the paper is still submerged in buffer
2. In a clean container or Incubation Tray, soak the E-PAGE™ Blotting Pad in 2X NuPAGE® Transfer Buffer (page 21). Inspect the pad for air bubbles. Press the pad to eliminate any visible air bubbles. **Note:** The Blotting Pad has no specific orientation (either side may be facing the gel).
3. Place 2 pieces of pre-soaked 2.5 mm Blotting Filter Paper from Step 1 on the anode plate of a semi-dry blotting apparatus. Ensure that all filter paper sheets are aligned properly and remove any air bubbles with the Blotting Roller.
4. Place the pre-soaked blotting membrane (see previous page) on top of the filter paper stack and remove any air bubbles with the Blotting Roller.
5. Remove the gel from the transfer buffer. Gently rub a gloved finger over the well side of the gel to remove small gel pieces from the gel surface. Re-submerge the gel in transfer buffer to remove any gel pieces.
6. Place the flat side of the gel on top of the blotting membrane (well side up), and remove any air bubbles with the Blotting Roller. Fill the wells of the gel with 2X NuPAGE® Transfer Buffer (page 21).
7. Place the pre-soaked E-PAGE™ Blotting Pad on the gel and gently roll out air bubbles with the Blotting Roller.
8. Place 2 pieces of pre-soaked 2.5 mm Blotting Filter Paper from Step 1 on top of the Blotting Pad. Ensure that all filter paper sheets are aligned properly and flush with the gel/membrane sandwich. Remove any air bubbles trapped between sheets using the Blotting Roller.
9. Place the cathode plate on the stack without disturbing the blot sandwich. Follow the manufacturer's instructions to further assemble the semi-dry blotting apparatus.
10. Transfer at 25 V for 1 h (~19 V/cm). You may need to optimize the transfer conditions for your specific proteins or semi-dry blot apparatus.

Using the E-Holder™ Platform

Introduction

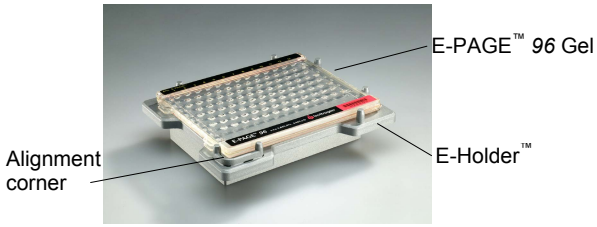
The E-Holder™ Platform is designed to hold E-PAGE™ *96* Gels during loading. Use the E-Holder™ when you need to load multiple gels while the other gels are running on the E-Base™

Note: The E-Holder™ is not a power supply unit, cannot be connected to an electrical outlet, and cannot be used to run E-PAGE™ *96* Gels.

To obtain the best results, run E-PAGE™ *96* Gels on the Mother E-Base™ or Daughter E-Base™ within 15 minutes after loading the gel on E-Holder™.

Procedure

1. Open the package and remove the gel.
2. Remove the comb from the E-PAGE™ *96* cassette.
3. Place the E-PAGE™ *96* cassette in the E-Holder™. Align the bottom left end of the cassette in the lower left alignment corner of the E-Holder™ as shown below. Proceed to loading the gel or to Step 4 for automated liquid handling system loading.



Note: The E-PAGE™ *96* Gel will not fit into the E-Gel® *96* Holder previously available from Invitrogen due to the tabs on the E-PAGE™ *96* cassette.

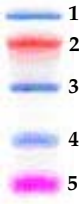
4. **Optional:** Set up your automated liquid handling system to load samples into the gel placed on an E-Holder™. Program your system to load the samples approximately 5 minutes before the previous electrophoresis run is complete. This will ensure that the loaded gel on the E-Holder™ will be placed onto a Mother E-Base™ or Daughter E-Base™ within the recommended time of 15 minutes.

Expected Results

Molecular Weight Calibration

The apparent molecular weight values shown below are derived from the construction of a calibration curve in the E-PAGE™ *96* buffer system. Use the values listed in the figures below for the most accurate calibration of your protein on an E-PAGE™ *96* Gel.

E-PAGE™ SeeBlue® Pre-stained Protein Standard Molecular Weight on E-PAGE™ *96* 6% Gel



1. 261 kDa
2. 173 kDa
3. 97 kDa
4. 42 kDa
5. 21 kDa

Chemiluminescent image of E-PAGE™ MagicMark™ Western Protein Standard

1. 220 kDa
2. 120 kDa
3. 60 kDa
4. 40 kDa
5. 20 kDa

Continued on next page

Expected Results, Continued

Electrophoresis Results

Electrophoresis results obtained using an E-PAGE™ *96* 6% Gel are shown below

E-PAGE™ SeeBlue® Pre-stained Protein Standard (10 µl) was loaded into alternate sample wells and 8 marker wells. The gel was electrophoresed for 14 minutes using standard conditions described in this manual.

Note: The wells of an E-PAGE™ *96* Gel are staggered. Protein bands migrate between adjacent wells in the row below. For example, the bands of lane A3 will migrate between wells B2 and B3.

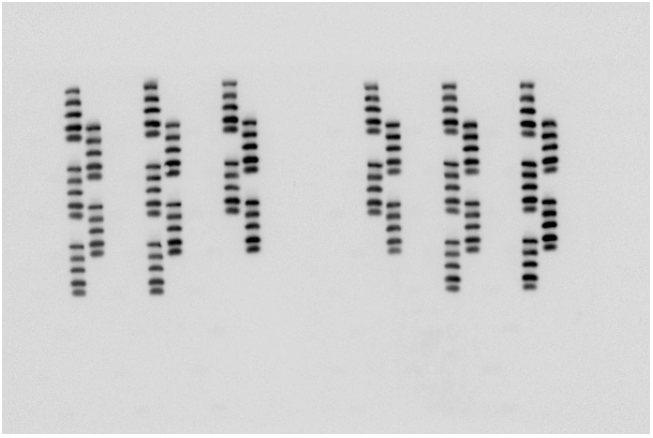
Continued on next page

Expected Results, Continued

Western Blotting Results

Western blotting results obtained using an E-PAGE™ *96* 6% Gel are shown below.

E-PAGE™ MagicMark™ Unstained Protein Standard (5 µl) was loaded into alternating sample wells. The gel was electrophoresed for 14 minutes using standard conditions described in this manual. Proteins were transferred to a 0.45 µm nitrocellulose membrane by semi-dry blotting as described. Detection was performed with WesternBreeze® Anti-Mouse Immunodetection Kit using 1:5000 dilution of Anti-V5 primary antibody from Invitrogen.



Continued on next page

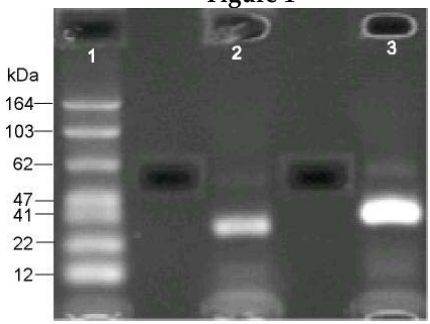
Expected Results, Continued

Results with Lumio™ Green Reagent

Results with Lumio™ detection on an E-PAGE™ *96* 6% Gel using the Lumio™ Green Detection Kit are shown below.

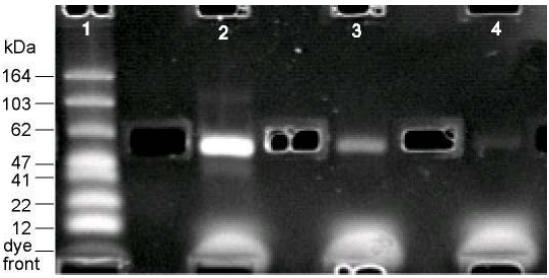
The gel in Figure 1 was visualized and imaged on a UV transilluminator (AlphaImager™ Imaging System) with an ethidium bromide filter using a 2 second exposure, and the gel in Figure 2 was visualized using a 4 second exposure.

Figure 1



Lane:	Sample:
1	BenchMark™ Fluorescent Protein Standard (5 µl)
2	*E. coli* CAT Lumio™ fusion protein (10 µl)
3	*E. coli* kinase Lumio™ fusion protein (10 µl)

Figure 2



Lane:	Sample:
1	BenchMark™ Fluorescent Protein Standard (5 µl)
2	48 kDa Lumio™ fusion protein (25 pmole)
3	48 kDa Lumio™ fusion protein (5 pmole)
4	48 kDa Lumio™ fusion protein (1 pmole)

Using E-Editor™ 2.02 Software

Introduction

E-Editor™ 2.02 software for Windows® allows you to reconfigure digital images of E-PAGE™ *96* Gels for analysis and documentation. E-Editor™ 2.02 reconfigures the wells of an E-PAGE™ *96* Gel into a side-by-side format for easy comparison and analysis.

You can reconfigure gels that were scanned in the original gel cassette, or gels that were removed from the cassette and stained or blotted. You can also group the images of multiple gels loaded from a 384-well microtiter plate into a single image with a layout corresponding to that of the original plate.

Capture an image of the gel as described below and use the E-Editor 2.02 software to:

- Align and arrange the lanes in the image
- Save the reconfigured image for further analysis
- Copy and paste selected lanes or the entire reconfigured image into other applications for printing, saving, emailing, and/or publishing

Guidelines for Imaging

Guidelines are provided below to image your E-PAGE™ *96* Gel after staining for analysis with E-Editor™ 2.02 software.

- Use a flatbed scanner or digital camera to capture a digital image of your E-PAGE™ *96* gel
- Align the gel properly while imaging (i.e., not at an angle), and make sure that the gel features are clear and distinct in the image.
- Image the gel using a resolution of **150 dpi** or higher.
- Scan the gel cassette well side up, if the gel is inside the cassette.
- Scan the gel well side up and fill the wells with water, if the gel is removed from the cassette to minimize interference from the wells in the image.
- Use a flatbed scanner with top illumination for best results.

Troubleshooting

Trouble-shooting

The table below provides some solutions to possible problems you might encounter during the electrophoresis of E-PAGE™ *96* Gels.

For troubleshooting problems with E-Base™ and staining, refer to the manuals supplied with the appropriate products.

Problem	Cause	Solution
No current	Daughter E-Base™ used without a Mother E-Base™	Do not use the Daughter E-Base™ without a Mother E-Base™. The Daughter E-Base™ does not have an electrical plug to connect to an electrical outlet.
	Copper contacts in the base are damaged due to improper use	Make sure that the copper contacts in the base are intact.
	Expired or defective gel cassette used	Use properly stored gels before the specified expiration date.
	E-PAGE™ *96* cassette is not correctly inserted into base	Remove cassette and reinsert. When the cassette is correctly inserted and power is on, a fan in the base will begin to run and a steady red light will be illuminated on the base.
Poor resolution or smearing of bands	Sample is overloaded	Do not load more than 20 µg of protein sample per well. For in-gel staining, load at least 200 ng protein per band.
	Very low volumes of sample were loaded	Load the recommended sample volume of 5-20 µl and always load 10-20 µl deionized water in all wells prior to sample loading. Avoid introducing bubbles while loading the samples. Bubbles will cause band distortion. For proper band separation, we recommend keeping sample volumes uniform and loading deionized water into empty wells.

Continued on next page

Troubleshooting, Continued

Problem	Cause	Solution
Poor resolution or smearing of bands	Incorrect loading buffer used	Use the recommended loading buffers as described on page 3.
	High salt or detergent concentration in samples	Be sure the final concentration of salt or detergent in the sample is as described on page 5. You may need to manually increase the run time for high salt or detergent samples to obtain optimal results.
	Gel was not electrophoresed immediately after sample loading	For best results, the gel should be run within 15 minutes of sample loading.
	A1 tip not aligned	Be sure to align the A1 tip properly prior to automated loading of E-PAGE™ Gels (page 10).
	Expired gel used	Use properly stored gels before the specified expiration date.
Sample leaking from the wells	Sample is overloaded or wells are damaged	Be sure to load the recommended volume of sample per well (page 5). Remove the comb carefully without damaging the wells.
Over-run the gel or need more time to run gel	Accidentally selected an incorrect program	Select program EP for E-PAGE™ *96* Gels. If you accidentally selected an incorrect program and are at the beginning of the run, stop the run and select the desired program. If you are well into the run, check the gel to see where the loading dye is running. Estimate the amount of time remaining and then manually stop the run.

Continued on next page

Troubleshooting, Continued

Problem	Cause	Solution
Protein bands distorted on membrane after semi-dry blotting	Non-uniform electric field created around wells	Be sure that the E-PAGE™ Blotting Pad is used correctly.
	Incorrect gel orientation	Be sure that the well side of the gel is not facing the membrane.
	Blot stack disturbed during or after assembly	Take care not to disturb the blot stack.
Weak transfer of high molecular weight samples during semi-dry blotting	Not enough SDS in sample	Make sure transfer buffer contains no methanol for transferring E-PAGE™ *96* Gels.
Weak transfer of low molecular weight samples	Use of large pore membranes allow small proteins to "blow through"	Use 0.2 µm nitrocellulose membrane for optimal capture of small proteins.

Appendix

Accessory Products

Additional Products

Ordering information for additional products available separately from Invitrogen is provided below. For details, contact Technical Service (next page) or visit www.invitrogen.com.

Product	Quantity	Catalog no.
Mother E-Base™	1	EB-M03
Daughter E-Base™	1	EB-D03
E-PAGE™ *48* Gels	8/pk	EP048-08
E-PAGE™ *48* Starter Kit	1 kit	EPST48-08
E-Holder™ Platform	2	EH-03
E-PAGE™ Loading Buffer 1 (4X)	4.5 ml	EPBUF-01
E-PAGE™ Blotting Pad	4/pk	LC2101
NuPAGE® Sample Reducing Agent (10X)	10 ml	NP0009
E-PAGE™ SeeBlue® Pre-stained Protein Standard	500 µl	LC5700
E-PAGE™ MagicMark™ Unstained Protein Standard	250 µl	LC5701
BenchMark™ Fluorescent Protein Standard	125 µl	LC5928
NuPAGE® Transfer Buffer (20X)	125 ml	NP0006
NuPAGE® Antioxidant	15 ml	NP0005
Blotting Roller	1	LC2100
Nitrocellulose/Filter Paper Sandwich, 0.45 µm	16/pk	LC2006
Nitrocellulose/Filter Paper Sandwich, 0.2 µm	16/pk	LC2009
Invitrolon™ PVDF/Filter Paper Sandwich, 0.45 µm	16/pk	LC2007
Blotting Filter Paper (2.5 mm)	50/pk	LC2008
Incubation Trays	8/pk	LC2102
Lumio™ Green Detection Kit	1 kit	LC6090
Large Gel Drying Kit	1 kit	NI2207
Gel-Dry™ Drying Solution (1X)	500 ml	LC4025
WesternBreeze® Chromogenic Kit Anti-Mouse	1 kit	WB7103
WesternBreeze® Chemiluminescent Kit Anti-Mouse	1 kit	WB7104

Technical Service

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- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your Web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact Us

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

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MSDS Information

To request an MSDS, visit our Web site at www.invitrogen.com. On the home page, go to 'Technical Resources', select 'MSDS', and follow instructions on the page.

Continued on next page

Technical Service, Continued

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Purchaser Notification, Continued

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