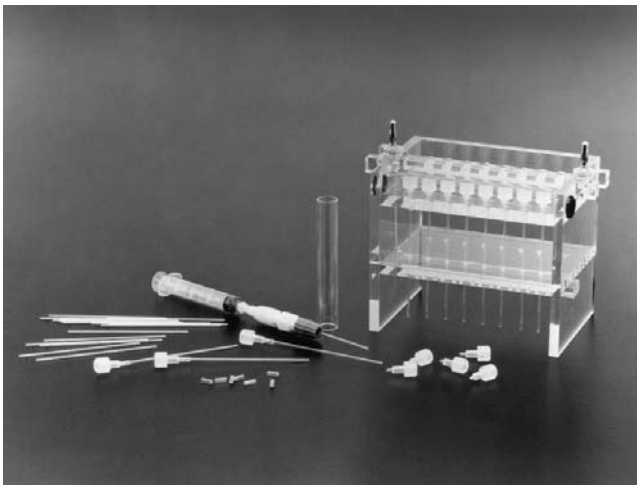


Mini-PROTEAN® 2-D Electrophoresis Cell, Mini-PROTEAN Tube Cell, and Mini-PROTEAN Tube Module

Instruction Manual

Catalog Numbers
165-2960,
165-2961 and 165-2965

BIO-RAD

Table of Contents

Note

To insure best performance from the Mini-PROTEAN 2-D electrophoresis cell, become fully acquainted with these operating instructions before using the cell to separate samples. Bio-Rad recommends that you first read these instructions carefully. Then assemble and disassemble the cell completely without casting a gel. After these preliminary steps, you should be ready to cast and run a gel.

Bio-Rad also recommends that all Mini-PROTEAN 2-D cell components and accessories be cleaned with a suitable laboratory cleaner (such as Bio-Rad Cleaning Concentrate, catalog number 161-0722) and rinsed thoroughly with distilled water, before use.

Model_____

Catalog No._____

Date of Delivery_____

Serial No._____

Invoice No._____

Purchase Order No._____

Warranty

Bio-Rad Laboratories warrants the Mini-PROTEAN 2-D cell against defects in materials and workmanship for 1 year. If any defects occur in the instrument during this warranty period, Bio-Rad Laboratories will repair or replace the defective parts free. The following defects, however, are specifically excluded:

1. Defects caused by improper operation.
2. Repair or modification done by anyone other than Bio-Rad Laboratories or an authorized agent.
3. Use of fittings or other spare parts supplied by anyone other than Bio-Rad Laboratories.
4. Damage caused by accident or misuse.
5. Damage caused by disaster.
6. Corrosion due to use of improper solvent or sample.

This warranty does not apply to parts listed below:

1. Platinum wire, glass plates.

For any inquiry or request for repair service, contact Bio-Rad Laboratories after confirming the model and serial number of your instrument.

Section 1
Introduction

The benefits of performing 2-D electrophoresis in a mini-format lie primarily in the reduced time required to complete the entire run. The IEF dimension can be completed in 2-4 hours. The equilibration step is eliminated or minimal, and the slab run takes only 35-45 minutes. Using Bio-Rad's protein stains (Bio-Safe Coomassie, SYPRO Ruby protein stain, Silver stain), the whole 2-D experiment can be completed in 1 day. Furthermore, the amount of reagents and sample used is significantly reduced.

This manual describes the procedure for preparing and running the O'Farrell first-dimension tube gels. For tips on sample preparation, and for alternative gel and buffer reagent preparation, refer to the FAQs available in the technical support section of our web site (http://discover.bio-rad.com). Refer to the Mini-PROTEAN slab cell instruction manual for details on performing the second-dimension SDS-PAGE electrophoresis.

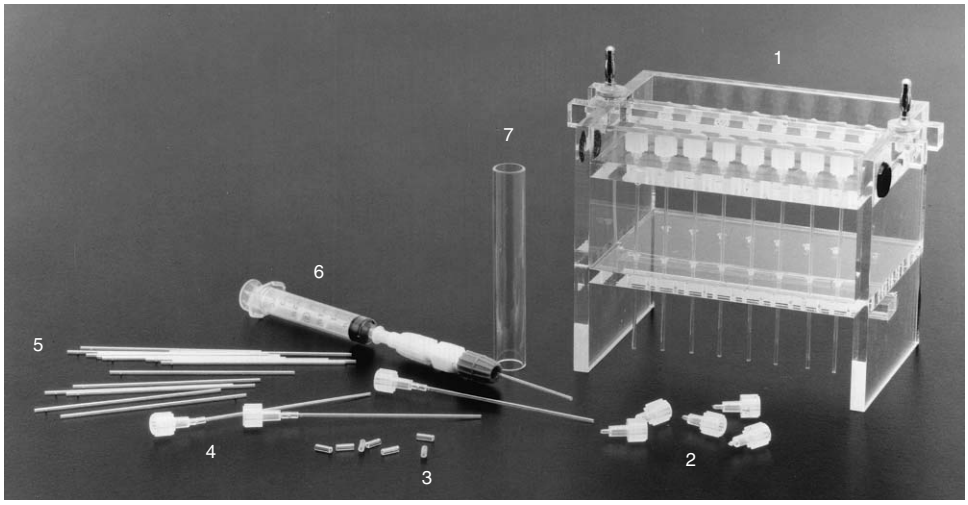
Section 2
Equipment and Reagents

2.1 Equipment

Catalog Number	Product Description
165-2960	**Mini-PROTEAN 2-D Cell,** includes tube cell module, 16 sample reservoirs and stoppers, 50 sample reservoir/capillary tube connectors, 200 capillary tubes with casting tube, tube gel ejector, Mini-PROTEAN electrophoresis module, 2 clamp assemblies, 10 sets of glass plates with 1.0 mm spacers, 2-D combs with 1 standard well (2), casting stand with gaskets, alignment card, leveling bubble, lid with power cables, and instructions.
165-2961	**Mini-PROTEAN Tube Cell,** includes tube cell module, 16 sample reservoirs and stoppers, 50 sample reservoir/capillary tube connectors, 200 capillary tubes with casting tube, tube gel ejector, lower buffer chamber, lid and instructions.
165-2965	**Mini-PROTEAN Tube Cell Module,** same as 165-2961 without lower buffer chamber and lid.

Warning:

Do not use alcohol to clean the plastic parts of the Mini-PROTEAN cell. This will cause damage to the acrylic components. Brief exposure to 10% ethanol at room temperature is exceptable. If you have questions about a specific chemical compatibility, please call Bio-Rad's technical support team (1-800-4BIORAD)



Mini-PROTEAN tube cell (catalog # 165-2961). Includes tube cell module (1), sample reservoirs (2), capillary tube connectors (3), tube setup; sample reservoirs/capillary tube connector/capillary tube (4), capillary tubes (5), tube gel ejector (6), and casting tube (7).

2.2 Description of Major Parts

The Mini-PROTEAN 2-D cell (catalog number 165-2960) consists of the following major parts:

1. Mini-PROTEAN electrophoresis cell for slab gels - A separate manual for the Mini-PROTEAN cell is included. Refer to it for a complete description of the slab cell parts.
2. Tube cell module - The tube cell module serves as the upper buffer chamber and holds up to 16 tubes. The module comes with 16 plastic stoppers to plug any tube holes not in use.
3. Sample reservoirs - The plastic sample reservoirs are molded with Luer tapered ends which fit tightly into the 16 tube holes in the tube cell module. Gel tubes are attached to the reservoirs with flexible Tygon tubing connectors.
4. Flexible tubing connectors (50) - These connectors are small pieces of Tygon tubing about 7.5 mm long and 2 mm in outer diameter. They are used to connect the sample reservoirs to the capillary gel tubes.
5. Capillary tubes (200) - The glass capillary tubes are used for running the first dimension tube gels.
6. Casting tube - The casting tube is about 8.5 cm long and 1.3 cm in diameter. It is used as a casting chamber to cast up to 40 capillary tube gels at once.
7. Tube gel ejector - The tube gel ejector is a green and white fitting which holds the glass capillary tubes firmly at one end, without leaking, so that the gels can be easily extruded. A series of O-rings in the fitting hold the tube tightly when the gel ejector is used as described. The gel ejector must be used with a 1.0 ml syringe.

2.3 Additional Required Equipment

The following additional material is required:

1. 1 ml and 10 ml syringes
2. Sample loading syringes, use a 20–100 µl Hamilton syringe because sample volumes can go up to 100 µl, sample reservoir accommodates a maximum of 200 µl of solution).
3. Intramedic tubing, 0.011" x 0.024" (American Scientific Products catalog number T6065-1)
4. Parafilm laboratory film
5. Tube gel casting stand (catalog number 165-2020) or ring stand and clamp, to hold the casting tube vertical
6. Power supply: Bio-Rad's PowerPac 3000 or 1000 power supply are recommended.
7. Combs and 1.0 mm spacers are needed for the second-dimension. These parts are supplied with the Mini-PROTEAN 2-D cell (catalog number 165-2960), but must be purchased separately if the tube cell or tube cell module (catalog numbers 165-2961 or 165-2965) is being used.

2.4 Accessories for the Mini Electrophoresis System

Catalog Number	Product Description
165-2966	**Capillary tubes (200) with casting tube**
165-2967	**Mini 2-D tube gel ejector**
165-2968	**Mini-PROTEAN Tube Gel sample reservoirs,** 8
165-2969	**Mini-PROTEAN Tube module stoppers,** 8
165-2970	**Mini-PROTEAN Tube module capillary tube connectors,** 50
170-3930	**Mini Trans-Blot® Electrophoretic Transfer Cell,** includes 2 gel holder cassettes, 4 fiber pads, 1 package of precut blot absorbent filter paper (thick), modular electrode assembly, Bio-Ice cooling unit, lower buffer chamber, lid with power cables, instructions.
170-3935	**Mini Trans-Blot Module,** same as 170-3930 without lower buffer chamber and lid.
165-2976	**Model 422 Electro-Eluter,** includes electro-eluter module, 12 Membrane Caps (molecular weight cut off of 12,000-15,000 daltons), 6 Glass Tubes with Frits, 6 Silicone Adaptors, 6 Grommets and Stoppers, lower buffer chamber with lid and cables, and instructions.
165-2977	**Model 422 Electro-Eluter Module,** same as 165-2976 without lower buffer chamber and lid.

Section 3
Solutions

3.1 Solutions for First-Dimension

1. **First-dimension acrylamide stock solution**

   Acrylamide/bis (30% T/5.4% C)

Acrylamide	28.38 g
Bis-acrylamide	1.62 g
	100 ml

   Fill to a total volume of 100 ml with distilled water. Filter and store at 4 °C in the dark (30 days maximum).

2. **10% Triton X-100 stock (w/v) solution:** Dilute 10 g Triton X-100 detergent to a total volume of 100 ml with distilled water. Deionize overnight with 5 gm of AG 501-X8 ion exchange resin. Conductivity should be ≤ 1 µ mho.

3. **First-dimension sample buffer**

8.0 M urea	5.7 g
2.0% Triton X-100	2.0 ml 10% Triton X-100 stock
5% β-mercaptoethanol	0.5 ml
1.6% Bio-Lyte 5/7 ampholyte	400 µl
0.4% Bio-Lyte 3/10 ampholyte	100 µl

   Dilute to 10 ml with distilled water (warm slightly in a water bath to dissolve urea). Do not exceed 30°C or the urea will break down. Aliquot into 0.5 ml volumes in Eppendorf tubes. Store at -70°C.

4. **First dimension sample overlay buffer**

4.0 M urea	5.41 g
0.8% Bio-Lyte 5/7 ampholyte	200 µl
0.2% Bio-Lyte 3/10 ampholyte	50 µl
Bromophenol blue	500 µl of a 0.05% (w/v) Bromophenol blue stock solution

   Dilute to 10 ml with distilled water. Warm in a water bath at no hotter than 30°C to dissolve the urea. Aliquot into 0.5 ml volumes in Eppendorf tubes. Store at -70°C.

5. **Upper Chamber Buffer (100 mM NaOH)**

   Dissolve 1.0 g NaOH in 250 ml distilled water and degas thoroughly for 30 minutes. Degassing helps prevent bubbles from forming in the sample reservoirs, or in the tubes between the gel and the sample reservoir. If bubbles form, electrophoresis is inhibited.

6. **Lower Chamber Buffer (10 mM H_3PO_4)**

   Dilute 1.36 ml concentrated H_3PO_4 in 2 L distilled water and degas thoroughly for 30 minutes. Degassing helps prevent bubbles from lodging on the bottom of the tubes.

3.2 Preparation of First-Dimension Tube Gels

1. **First-dimension gel monomer solution**

8.0 M urea	5.5 g
4% acrylamide (total monomer)	1.33 ml acrylamide stock
2% Triton X-100	2.0 ml 10% Triton X-100
1.6% Bio-Lyte 5/7 ampholyte	0.400 ml Bio-Lyte 5/7 ampholyte
0.4% Bio-Lyte 3/10 ampholyte	0.100 ml Bio-Lyte 3/10 ampholyte
	1.97 ml distilled water
0.01% ammonium persulfate	10 µl 10% ammonium persulfate (make this solution fresh daily)
0.1% TEMED	10 µl TEMED

This makes 10 ml total volume, enough to cast one set of gels using the casting tube. Make this solution fresh. Weigh out the urea. Add all other ingredients except the last two, which are the polymerization catalysts. Dissolve the urea by warming the vessel in warm water (≤ 37°C) with swirling. Degas the solution thoroughly for 15 minutes. Add the APS and TEMED, and swirl gently and briefly. Cast the gels as directed in Section 5.

3.3 Solutions for Second-Dimension

1. **SDS sample equilibration buffer**

0.0625 M Tris HC1, pH 6.8	12.5 ml 0.5 M Tris/HCl, pH 6.8
2.3% (w/v) SDS	23 ml 10% (w/v) SDS
5.0% (v/v) β-mercaptoethanol	5 ml
10% glycerol (w/v)	8 ml
Bromophenol blue	2.5 ml 0.05% (w/v) stock solution
Distilled water	49 ml
	100 ml

2. **1% agarose in SDS sample buffer (optional, refer to Section 7.2)**

   Prepare SDS equilibration buffer without β-mercaptoethanol. Melt 1 g agarose/100ml.
   Add β-mercaptoethanol to 5%.

3. **Solid molecular weight standard preparation**

   Prepare a 1:100 dilution of molecular weight standard in molten 1% agarose in SDS equilibration buffer. Cast a standard agarose gel in a mini 2-D tube gel. Let sit at room temperature, and extrude the gel onto Parafilm laboratory film. Cut into 0.6 cm lengths. The gel slices can be stored in Eppendorf tubes at -20° C.

   Volume of the 0.6 cm molecular weight standard $= \pi r^2 \times \text{length}$
   $= \pi\ (0.05\ \text{cm})^2 \times 0.6\ \text{cm}$
   $\approx 5\ \mu\text{l}$

   For second dimension gel solutions, refer to the Mini-PROTEAN cell instruction manual.

Section 4
Safety Instructions

Power to the Mini-PROTEAN tube cell is supplied by an external DC voltage power supply. **This power supply must be ground isolated in such a way that the DC voltage output floats with respect to ground.** All of Bio-Rad's power supplies meet this important safety requirement. Regardless of which power supply is used, the maximum specified operating parameters for the cell are:

1500 VDC ⎓	maximum voltage limit
5 Watts	maximum power limit
50 °C	maximum ambient temperature limit

Current to the cell, provided from the external power supply, enters the unit through the lid assembly, providing a safety interlock to the user. Current to the cell is broken when the lid is removed. **Do not attempt to circumvent this safety interlock, and always turn the power supply off before removing the lid, or when working with the cell in any way.**

Since potentially dangerous voltages may be used in the electrophoresis cell, it is important that certain precautions are observed for the safety of the operator. Please become familiar with these precautions, both for safe operation of this equipment and to prevent the loss of valuable experimental results.

1. Always turn off the power supply before removing the safety cover of the electrophoresis cell. Do not use the safety cover as an on-off switch.
2. Never attempt to operate the electrophoresis cell without the safety cover.
3. Do not attempt to move the electrophoresis cell with the power on.
4. Do not leave an operating electrophoresis cell unattended for long periods. Overnight runs at high voltages are to be avoided. Overnight runs at voltages up to 400 V are acceptable.
5. Do not overfill the upper or lower buffer reservoirs. This can cause current leaks and arcing. Refer to Section 6.1 for proper buffer levels.
6. Insure that the upper horizontal surfaces of the tube gel module are dry before starting IEF separation, especially when high voltages are used.
7. If the electrophoresis cell appears to be drawing excessive current, or if the current fluctuates, turn off the power supply and check the entire system (for example, buffer chamber or sample reservoir leaks, overfilled buffer chamber, buffer too conductive, etc.).
8. Never allow untrained personnel to use this equipment without proper supervision.

Important

This Bio-Rad instrument is designed and certified to meet IEC1010-1* safety standards. Certified products are safe to use when operated in accordance with the instruction manual. This instrument should not be modified or altered in any way. Alteration of this instrument will:

- Void the manufacturer's warranty
- Void the IEC1010-1 safety certification
- Create a potential safety hazard

Bio-Rad is not responsible for any injury or damage caused by the use of this instrument for purposes other than for which it is intended or by modification of the instrument not performed by Bio-Rad or an authorized agent.

*IEC1010-1 is an internationally accepted electrical safety standard for laboratory instruments

Section 5
The First-Dimension (IEF)

5.1 First-Dimension Tube Gels

Careful casting of the tube gels is extremely important for gel to gel reproducibility. If the tube gels are not the same length, then the R_m values for the IEF dimension will vary from gel to gel, and it will be difficult to compare spots on different gels.

Casting and running two or three gels per sample is recommended, in case some gels are lost during the procedure. It is also suggested that you go through the procedure once or twice with standards or samples not essential to your research program, to become familiar with the procedure.

Note: A minimum of 4–8 tubes must be run at a time. The exact minimum number of tubes depends on the power supply, and it's capability to read and maintain low amperage.

5.2 Casting Tube Gels

1. Seal one end of the casting tube with several layers of Parafilm laboratory film.
2. Fill the casting tube with capillary gel tubes so that the blue stripped ends of the gel tubes are at the open end of the casting tube. The capillary gel tubes must be kept vertical. This is best achieved by filling the casting tube full with capillary gel tubes. A glass rod can be used to take up space, if necessary.
3. Prepare a clamp and ring stand or a tube rack (catalog #165-2020) to maintain the casting tube in a vertical position. The casting tube will also stand on a benchtop without support, although it is much less stable. In any case, the casting tube must be kept vertical and level to obtain reproducible tube gels.

4. After adding the catalysts, fill a syringe with monomer solution, and attach a needle.

   Caution: Wear gloves when handling acrylamide!

5. Insert the needle into the casting tube so that the point is below the tops of the capillary gel tubes. Deliver monomer solution so that the gel tubes fill from the bottom. Some bubbles will remain, but most bubbles will be between the tubes, rather than in them. Cast the gels to the top of the tubes. Tap the casting tube to remove trapped bubbles. Fix the casting tube in the vertical position with the clamp and ring stand, or place the casting tube in the tube rack. With the casting tube vertical, allow the monomer to polymerize. This process should take approximately one hour.

6. After polymerization, remove the Parafilm laboratory film slowly from the bottom of the casting tube, and push the batch of capillary tubes out of the casting tube from the top. Be careful not to introduce bubbles into the bottom of the tubes.

7. Wipe the extra acrylamide off the tubes. Do this carefully, to avoid introducing air bubbles into the top or bottom of the tubes. If a tube contains any bubbles it must be discarded, because the bubble breaks the electrical circuit, and electrofocusing will not occur.

8. Attach the blue stripped end of the tubes to the sample reservoirs with the flexible tubing connectors.

   Note: The tubing connectors must be replaced after each use. Repeated use will cause the connectors to become distorted so they no longer fit snugly over the ends of the capillary tubes or the sample reservoirs. Failure to replace worn tubing connectors can result in upper buffer leaks and potential damage to the tube adaptor.

9. Rinse the bottoms of the tubes with lower buffer.

Section 6
Running the First-Dimension (IEF)

6.1 Electrode Preparation

1. Insert a gel tube with reservoir or a stopper into each of the 16 positions in the tube adaptor. To insure that the upper buffer does not leak into the lower buffer chamber, the sample reservoirs and stoppers must be firmly inserted into the tapered holes in the tube adaptor. Insert the stoppers with a twisting motion.
2. Fill each sample reservoir with thoroughly degassed upper buffer chamber electrolyte. Using a sample loading syringe with needle, e.g., 20 µl Hamilton syringe, expel any air bubbles from the capillary space in the neck of the sample reservoir and in the flexible tubing connector.
3. Place a 1 inch stir bar in the lower chamber, and place the tube gel adaptor into the lower buffer tank. Fill the lower tank with electrolyte. The maximum buffer level in the lower buffer chamber should be enough to bring it about even with the blue line on the glass gel tubes. This corresponds to an approximate volume of 800 ml. If the buffer level is higher than the blue lines on the gel tubes, an electrical short circuit may be created, which will prevent electrofocusing and may damage the equipment. Check the bottom of each tube for trapped air bubbles. Bubbles can be removed using a Pasteur pipet with a curved tip.
4. Fill the upper buffer chamber of the tube adaptor module. The buffer level in the upper buffer chamber should be slightly above the sample reservoirs but below the top of the plastic bar under which the platinum electrode is mounted. This is a volume of approximately 60 ml. If the upper buffer level is too high, there may be an electrical short circuit created between the banana plug connector for the lower chamber and the liquid in the upper chamber. Before operating the unit, make sure there is no moisture around the banana plug connectors which might create a short circuit. Dry these areas with a paper towel if necessary.

   Note: It is important not to overfill the buffer chambers in the 2-D cell. If the chambers are overfilled, an electrical short circuit can occur with the upper chamber causing excessive current to be drawn from the power supply. This may damage the tube adaptor due to overheating.
5. Place the lid on the cell. Match the red lead on the lid with the red coded post on the tube adaptor.
6. Place the unit of a magnetic stirring motor, and stir slowly. This will provide cooling for the heating, which can occur in the capillary tubes.
7. Connect the cell to the power supply and pre-electrophorese the tube gels by running at 200 V for 10 minutes, 300 V for 15 minutes, and 400 V for 15 minutes. The Bio-Rad PowerPac 3000 and PowerPac 1000 power supplies are recommended for this application. These steps are performed automatically when the unit has been programmed for this operation.

   Note: Pre-electrophoresis, as described in steps 5, 6, and 7 of Section 6.1, is optional. The original O'Farrell protocol uses pre-electrophoresis. Other protocols indicate it is not required.

6.2 Sample Preparation

Add an equal volume of first-dimension sample buffer to the sample. Incubate at room temperature for 10–15 minutes. The sample reservoirs can accommodate volumes up to 100 µl, but smaller volumes are recommended, e.g., 25 µl. An optimum protein load for a complex sample is about 5–10 µg protein, though higher protein loads can be applied with satisfactory results. For purified proteins, the recommended amount is about 1–5 µg. If the spots are detected with silver stain, or SYPRO Ruby protein gel stain smaller amounts of sample may be satisfactory.

6.3 First-Dimension (IEF) Sample Loading

1. After pre-electrophoresis, discard the upper and lower chamber buffers. Remove the pre-electrophoresis solutions from the sample reservoirs and the tube gels with the Hamilton syringe. Fill the gel tubes and the sample reservoirs with freshly degassed upper chamber buffer. Remove all bubbles with the Hamilton syringe.
2. To load sample, use a sample syringe with a blunt needle. Add sample at the top surface of the gel.
3. Overlay the sample with 20-40 µl sample overlay buffer. This buffer must have an intermediate density between the sample and the electrolyte. It may be necessary to dilute the sample overlay buffer with a small volume of distilled water.
4. Set up the apparatus as before (Section 6.1 steps 4–8). Do not disturb the sample while filling the upper buffer chamber with electrolyte solution.

6.4 Power Conditions

1. Run at 500 V for 10 minutes.
2. Increase the voltage to 750 V for 3.5 hours. Higher voltages are not recommended because the unit may overheat.

   Note: If the first-dimension (IEF) run is done at lower voltages, the run time must be increased. For example, when IEF is done at 400 V the run will be completed in approximately 6 hours. The times given here are approximate and the IEF run time must be determined for each sample. To determine the optimum IEF run time at a given voltage, set up eight tube gels with the same sample. Remove two tubes every 2 hours, and run the tube gels in the second dimension according to the directions below. A comparison of the results should provide the information needed to determine the optimum IEF run time.

Section 7
The Second-Dimension

While the first-dimension tube gels are running, cast the second dimension slab gels according to the directions in the Mini-PROTEAN cell instruction manual. For reproducibility of slab gel composition of multiple gels, use the Mini-PROTEAN multi-gel casting chamber.

7.1 Preparation of the Stacking Gel

1. To prepare the stacking gel with a standard well for the second-dimension run, use the 2-D comb pushed halfway in. Cast a narrow stacking gel (maximum 5 mm), and fill the excess space on top of the tube gel for the equilibration buffer, if necessary. Follow the instructions in the Mini-PROTEAN cell instruction manual. Alternatively, when using molecular weight standards in agarose, cast the stacking gel and overlay with water over the entire surface of the gel without using a comb. Cast the molecular weight standards into an agarose tube gel as described in the solutions section, and load the agarose gel piece directly onto the stacker with a spatula after application of the first-dimension tube gel.

7.2 Removing Gel from the Tube and Loading onto the Slab Gel

1. After the first-dimension run is complete, turn off the power, disconnect the lid and remove the tube gel module. Separate the tubes from the connectors and sample reservoirs.
2. Attach a 1.0 ml syringe to the white end of the tube gel ejector. Draw up Laemmli electrophoresis buffer through the ejector into the syringe. (Preparation of the SDS-PAGE (Laemmli) running buffer is described in the Mini-PROTEAN cell manual.)
3. Loosen the colored end of the gel ejector by unscrewing it almost all the way. Separate the capillary tube from the sample reservoir. Insert the capillary tube into the colored end of the unit when it is unscrewed. The tube will encounter minor resistance and must be inserted all the way in to get a tight seal. When the tube is all the way in, tighten the colored part to secure the tube.
4. Put equilibration or reducing buffer between the slab gel plates so that the tube gel will slide easily between the glass plates.
5. Extrude the tube gel onto a piece of Parafilm laboratory film. When extruding the gel, pay attention to the amount of pressure applied to the plunger. Push hard on the plunger until the gel begins to move down the tube. Once the gel starts moving, the resistance decreases rapidly. When you notice that the gel has started to move, release the pressure and then push more slowly.
6. Wet the tube gel slightly with SDS sample equilibration buffer. With a clean spatula, straighten out the gel and position it lengthwise on the Parafilm laboratory film. Carefully slide the gel from the Parafilm laboratory film between the glass plates and onto the slab gel.

7. To equilibrate the tube gel, overlay it with enough SDS sample buffer to cover it completely, (about 200 µl), and allow it to sit for up to 10 minutes. After equilibration, overlay with running buffer. Fill the electrode buffer reservoirs, and begin the second-dimension run. An agarose overlay is recommended, but is not necessary in this procedure.

7.3 Running the Slab Gels

Instructions for casting and running mini slab gels are in the Mini-PROTEAN cell instruction manual. Suggestions for staining and destaining the slab gels are also in the Mini-PROTEAN cell manual.

Section 8
Maintenance of Equipment

Mini-PROTEAN tube adaptor	Rinse thoroughly with distilled water after every use.
Sample reservoirs **Flexible tubing connectors** **Casting tube**	After use, rinse with laboratory detergent solution, then rinse with distilled water.
Mini-PROTEAN electrophoresis cell	Refer to Mini-PROTEAN cell instruction manual.

Section 9
Troubleshooting Guide

1. No load or no current detected by the power supply. IEF gel runs with high voltage and low current. Typically, an IEF run will end with a current reading at or below 1 mAmp.

   a. Be sure that the IEF run includes at least 8 tubes, even if some of the tubes are blank (contain no sample). Make suret he blank tubes contain ampholytes.

   b. Check that no air bubbles are trapped at the top or bottom of the tube.

   c. Check the limitations of the power supply. Some power supplies will register an error message when the current goes below 1–10 mAmps because they cannot detect low current.

2. First-dimension gels do not focus well.
   a. Check the tube gels, tubing connectors, sample reservoir and ends of the gels for bubbles.
   b. Check the pH of the upper and lower chamber buffers. Increase the NaOH concentration of the buffer in the upper chamber.
   c. Pre-electrophorese the tube gels prior to loading samples.
   d. Increase the focussing time.
   e. Reduce the salt concentration in the sample.

3. Streaking in the second-dimension gels. (Refer to the Mini-PROTEAN cell instruction manual for further troubleshooting.)

   Horizontal streaking:
   a. Sample preparation problems. Add detergent containing sample buffer, then centrifuge at 100,000 x g to remove the insoluble pellet.
   b. Overloaded protein sample. Load less protein.
   c. Nucleic acids bound to the protein. Treat sample with an endonuclease.
   d. Focusing time not optimized. Perform a time course to determine the best volt-hours for the samples.

   Vertical streaking:
   a. Problems in the sample. Determine if the vertical streaks are related to the sample by running an SDS-PAGE molecular weight standard on the same gel.
   b. Pinpoint streaking in the background of the gel may be caused by dust or bacteria in the water supply. Filter all the solutions through a 0.45 µm nitrocellulose membrane.

	c. Vertical streaking that is connected to a protein spot may be caused by protein aggregation, incomplete reduction, or incomplete alkylation in the sample. Increase the SDS concentration in the agarose overlay (up to 2%) and/or in the SDS-PAGE running buffer (up to 0.4%). For incomplete reduction or alkylation, increase the concentration of reduction (DTT or TBP) or alkylation (iodoacetamide) reagents, or use fresh reagents.
4. Storing tube gels after IEF.	Extrude gel from the tube and store (with or without buffer) at -20°C or lower.
5. Further troubleshooting for SDS-PAGE gels.	Refer to the Mini-PROTEAN cell instruction manual.

Appendix
Electrophoresis Chemicals

Catalog Number	Product Description	Quantity/ Package
161-0100	**Acrylamide,** 99.9%	100 g
161-0101	**Acrylamide,** 99.9%	500 g
161-0107	**Acrylamide,** 99.9%	1 kg
161-0103	**Acrylamide,** 99.9%	2 kg
161-0122	**Preweighed Acrylamide/Bis,** 37.5:1 mixture	30 g
161-0125	**Preweighed Acrylamide/Bis,** 37.5:1 mixture	150 g
161-0200	**Bis (N,N'-Methylene-bis acrylamide)**	5 g
161-0201	**Bis (N,N'-Methylene-bis acrylamide)**	50 g
161-0716	**Tris**	500 g
161-0719	**Tris**	1 kg
161-0717	**Glycine**	250 g
161-0718	**Glycine**	1 kg
161-0300	**SDS (Sodium dodecylsulfate)**	25 g
161-0301	**SDS (Sodium dodecylsulfate)**	100 g
161-0302	**SDS (Sodium dodecylsulfate)**	1 kg
161-0700	**Ammonium Persulfate (APS)**	10 g
161-0610	**Dithiothreitol (DTT)**	1 g
161-0611	**Dithiothreitol (DTT)**	5 g
161-0710	**2-Mercaptoethanol**	25 ml
161-0800	**TEMED**	5 ml
161-0801	**TEMED**	50 ml
162-0100	**Agarose, Standard Low -m_r**	100 g
162-0102	**Agarose, Standard Low -m_r**	500 g
161-0304	**SDS-PAGE Standards,** Low MW	
161-0303	**SDS-PAGE Standards,** High MW	
161-0443	**Silver Stain Kit,** includes 1 bottle oxidizer concentrate, 1 bottle silver reagent concentrate, and 4 bottles developer. Enough to stain approximately 24 gels.	
161-0400	**Coomassie Blue R-250**	10 g
161-0404	**Bromophenol Blue**	10 g

Catalog Number	Product Description	Quantity/ Package
161-0407	**Triton X-100 Detergent**	500 ml
161-0730	**Urea**	250 g
161-0731	**Urea**	1 kg
161-0460	**CHAPS**	1 g
161-0465	**CHAPSO**	1 g

Bio-Lyte Ampholytes

163-1112	**Bio-Lyte 3/10 Ampholyte,** 40%	10 ml
163-1132	**Bio-Lyte 3/5 Ampholyte,** 20%	10 ml
163-1142	**Bio-Lyte 4/6 Ampholyte,** 40%	10 ml
163-1152	**Bio-Lyte 5/7 Ampholyte,** 40%	10 ml
163-1162	**Bio-Lyte 6/8 Ampholyte,** 40%	10 ml
163-1172	**Bio-Lyte 7/9 Ampholyte,** 40%	10 ml
163-1182	**Bio-Lyte 8/10 Ampholyte,** 20%	10 ml
142-6424	**AG 501-X8 Ion Exchange Resin**	500 g

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***Bio-Rad Laboratories Main Office*** *2000 Alfred Nobel Drive, Hercules, CA 94547, Ph. (510) 741-1000, Fx. (510) 741-5800*
Also in: ***Australia*** *Ph. 02 9914 2800, Fx. 02 9914 2889* ***Austria*** *Ph. (01) 877 89 01, Fx. (01) 876 56 29* ***Belgium*** *Ph. 09-385 55 11, Fx. 09-385 65 54*
Brazil *Ph. 55 21 507 6191* ***Canada*** *Ph. (905) 712-2771, Fx. (905) 712-2990* ***China*** *Ph. (86-21) 63052255, Fx. (86-21) 53964775*
Czech Republic *Ph. (420) 2-4141 0532 Fx. (420) 2-4143 1642* ***Denmark*** *Ph. 45 44 52-1000, Fx. 45 4452 1001*
Finland *Ph. 358 (0)9 804 2200, Fx. 358 (0)9 804 1100* ***France*** *Ph. 01 47 95 69 65, Fx. 01 47 41 9133* ***Germany*** *Ph. 089 318 84-177, Fx. 089 318 84-123*
Hong Kong *Ph. 852-2789-3300, Fx. 852-2789-1257* ***India*** *Ph. (91-124)-6398112/113/114, 6450092/93, Fx. (91-124)-6398115/6450095*
Israel *Ph. 03 951 4127, Fx. 03 951 4129* ***Italy*** *Ph. 39 02 216091, Fx. 39 02 21609399* ***Japan*** *Ph. 03-5811-6270, Fx. 03-5811-6272*
Korea *Ph. 82-2-3473-4460, Fx. 82-2-3472-7003* ***Latin America*** *Ph. 305-894-5950, Fx. 305-894-5960* ***Mexico*** *Ph. 52 5 534 2552 to 54, Fx. 52 5 524 5971*
The Netherlands *Ph. 0318-540666, Fx. 0318-542216* ***New Zealand*** *Ph. 64-9-4152280, Fx. 64-9-443 3097* ***Norway*** *Ph. 47-23-38-41-30, Fx. 47-23-38-41-39*
Poland *Ph. (48) 22-8126 672, Fx. (48) 22-8126 682* ***Portugal*** *Ph. 351-21-472-7700, Fx. 351-21-472-7777* ***Russia*** *Ph. 7 095 721 1404, Fx. 7 095 721 1412*
Singapore *Ph. 65-2729877, Fx. 65-2734835* ***South Africa*** *00 27 11 4428508, Fx. 00 27 11 4428525* ***Spain*** *Ph. 34 91 590 5200, Fx. 34 91 590 5211*
Sweden *Ph. 46 (0)8-55 51 27 00, Fx. 46 (0)8-55 51 27 80* ***Switzerland*** *Ph. 061 717-9555, Fx. 061 717-9550* ***United Kingdom*** *Ph. 0800-181134, Fx. 01442-259118*

Sig 0402

M1652960 Rev C