# Mini-PROTEAN® II Electrophoresis Cell

# Instruction Manual

BIO-RAD

For Technical Service Call Your Local Bio-Rad Office or in the U.S. Call **1-800-4BIORAD** (1-800-424-6723)

# Table of Contents

# Note

To insure best performance from the Mini-PROTEAN II cell, please become fully acquainted with these operating instructions before using the cell. Assemble and disassemble the cell completely without casting a gel to familiarize yourself with the components. You should then be ready to cast and run a gel.

Bio-Rad also recommends that all Mini-PROTEAN II 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.

**WARNING: Do not use alcohol to clean the plastic parts of the Mini-PROTEAN II cell. This will cause damage to the acrylic components.**

**Model** _____

**Catalog No.** _____

**Date of Delivery** _____

**Serial No.** _____

**Invoice No.** _____

**Purchase Order No.** _____

# Warranty

Bio-Rad Laboratories warrants the Mini-PROTEAN II 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.
2. Glass plates.

For any inquiry or request for repair service, contact Bio-Rad Laboratories. Please be able to provide the model and serial number of your instrument.

# Section 1
# General Information

## 1.1 introduction

The Mini-PROTEAN II electrophoresis cell allows rapid analysis of protein and nucleic acid samples in miniature polyacrylamide and agarose gels. The cell allows analysis to be completed two to three times faster than is possible with conventional 16 cm cells, while maintaining comparable resolution. The Mini-PROTEAN II cell can run two slab gels for analysis of up to 30 samples at one time.

The Mini-PROTEAN II cell makes casting and running miniature slab gels almost effortless. Glass plate sandwiches are assembled using the unique four-screw clamp assemblies, then transferred to the alignment slot of the separate casting stand. The clamp screws are loosened, the plates and spacers are aligned, the screws are re-tightened, and the glass plate sandwich assembly is snapped into one of the two casting slots of the casting stand in one simple motion. No grease or agarose plug is required for casting. When the gels are cast, the clamp assemblies are snapped onto the inner cooling care, samples are loaded, and the electrophoretic run is complete within 45 minutes. While two gels (up to thirty samples) are being run in the Mini-PROTEAN II cell, two more may be cast in the separate casting stand.

## 1.2 Specifications

**Constructions**

| | |
|---|---|
| **Inner cooling core** | Molded liquid crystal polymer |
| **Clamp assemblies** | Glass-filled liquid crystal polymer clamps with acrylic pressure plate |
| **Lower buffer chamber, lid** | Molded polycarbonate |
| **Casting stand** | Molded polycarbonate |
| **Electrodes** | Platinum wire 0.254 mm diameter |
| **Gaskets: cooling core, casting stand** | Silicone rubber |
| **Combs** | Teflon® |
| **Spacers** | Polyvinyl chloride |
| **Shipping weight** | 2.2 kg |
| **Overall size** | 16 cm (L) x 12 cm (W) x 18 cm (H) |
| **Gel size** | 7 cm (L) x 8 cm (W) |
| **Glass plate size** | (inner) 7.3 cm x 10.2 cm<br>(outer) 8.3 cm x 10.2 cm |
| **Voltage Limit** | 600 VDC |

**Note:** Mini-PROTEAN II cell components are not compatible with chlorinated hydrocarbons (e.g., chloroform), aromatic hydrocarbons (e.g., toluene, benzene), acetone, ethanol, or 2 amino-2 methyl-1, 3 propanediol. Use of such organic solvents voids all warranties. Call 1-800-4-BIORAD for technical information regarding chemical compatibility of Mini-PROTEAN II cell components with various laboratory reagents.

## 1.3 Safety

Power to the Mini-PROTEAN II cell is to be 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:

| | |
|---|---|
| **600 VDC ⎓** | maximum voltage limit |
| **15 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 use the cell without the safety lid, and always turn the power supply off before removing the lid, or when working with the cell in any way.

### 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 use of this instrument for purposes other than for which it is intended or by modifications 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 2
# Description of Major Parts

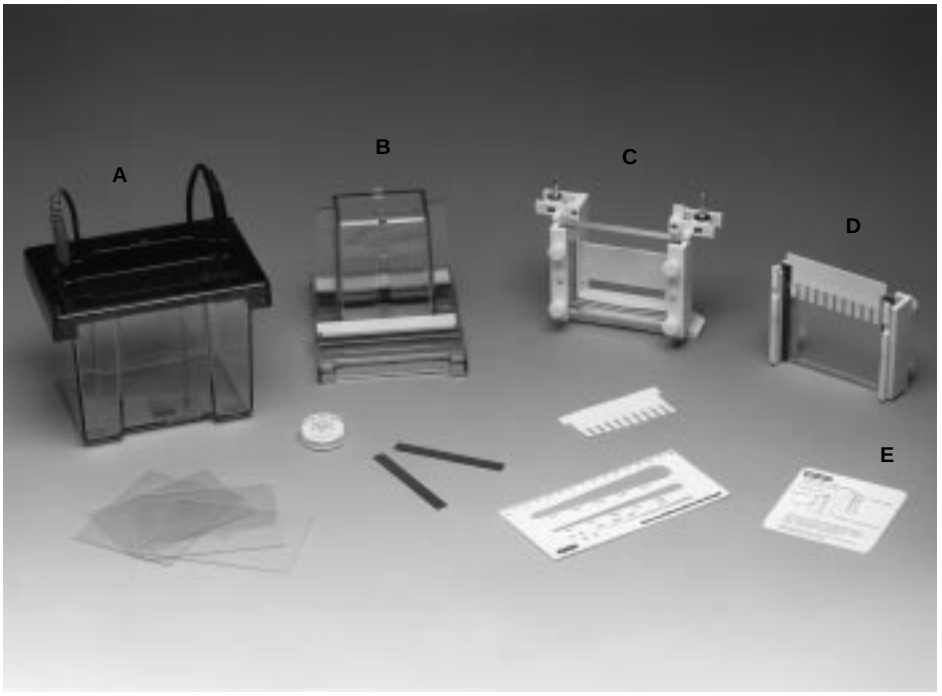



**Fig. 2.1 Mini-PROTEAN II electrophoresis cell, lower buffer chamber, and lid (a), casting stand (b), inner cooling core (c), and sandwich clamp assemblies (d), casting alignment card (e).**

## 2.1 Sandwich Clamp Assemblies

Sandwich clamp assemblies consist of two clamps with two thumbscrews each, permanently attached to a clear acrylic pressure plate. A gel sandwich is formed by placing a long (outer) plate against the acrylic pressure plate, adding two spacers, and then a shorter (inner) plate to complete the sandwich.

The combination of the four clamp screws and the acrylic pressure plate assures even pressure over the entire length of the glass plates and spacers, providing a leak-proof seal while preventing plate damage due to uneven pressure.

## 2.2 Casting Stand

The casting stand is separate from the Mini-PROTEAN II cell so that two gels may be cast while two others are being run. The stand consists of one alignment slot and two casting slots. The glass plates and spacers of the gel sandwich are aligned in the alignment slot of the casting stand and then the glass plate assembly is snapped into one of the casting slots. The rubber gasket provides a leak-proof seal without grease or an agarose plug.

## 2.3 Inner Cooling Core

After gels are cast, the clamp assemblies are snapped onto the inner cooling core to form the upper buffer chamber. The molded U-shaped gaskets provide leak-free seals without grease. The upper buffer is in direct contact with the inner glass plate of the gel sandwich to provide even heat distribution over the entire gel, preventing thermal band distortion during electrophoretic separations.

The inner cooling core houses both the upper and lower electrodes and the connecting jacks. The anode or lower buffer chamber jack is identified with a red marker and the cathode or upper chamber jack with a black marker.

## 2.4 Lower Buffer Chamber and Lid

The lower buffer chamber and lid combine to fully enclose the Mini-PROTEAN II cell during electrophoresis, providing electrical insulation. The lid cannot be removed without disconnecting the electrical circuit. This lid and buffer chamber can be used with a variety of different electrode modules to convert the Mini-PROTEAN II cell into a Mini Trans-Blot® cell, a mini 2-D electrophoresis cell, or an electro-elution cell for preparative recovery of proteins and nucleic acids.

# Section 3
# Assembling the Glass Plate Sandwiches

**Note:** To insure proper alignment and casting, make sure plates, spacers, combs, and casting stand gaskets are clean and dry before proceeding.

1. Assemble the gel sandwich on a clean surface. Lay the longer rectangular glass plate down first, then place two spacers of equal thickness along the short edges of the rectangular plate. Next, place the shorter glass plate on top of the spacers so that the bottom ends of the spacers and glass plates are aligned (Figure 3.1). At this point, the spacers should be sticking up about 5 mm above the long glass plate.

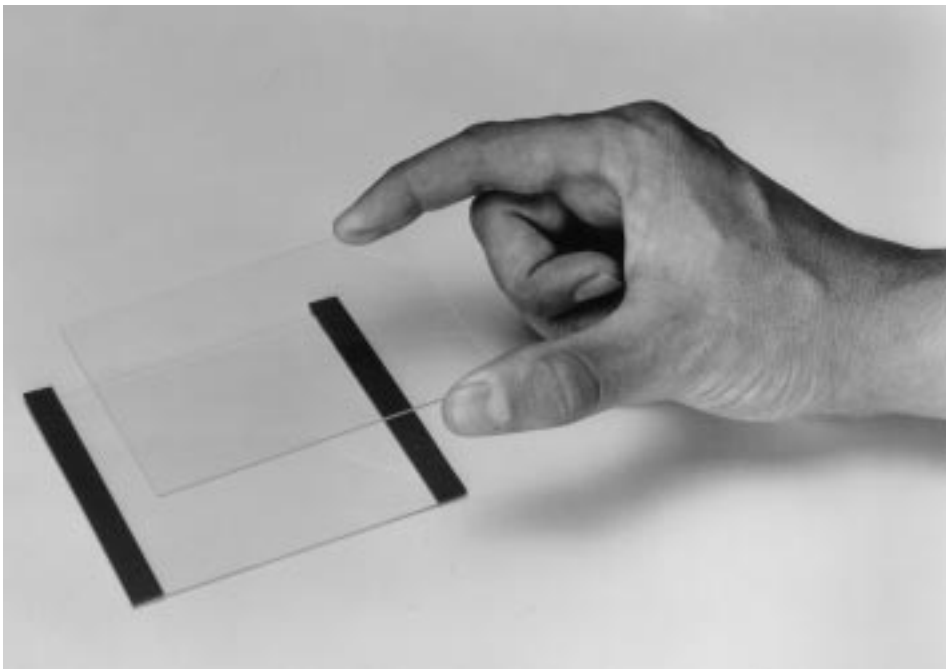


**Fig 3.1**

2. Loosen the four screws on the clamp assembly and stand it up so that the screws are facing away from you. Firmly grasp the glass plate sandwich with the longer plate facing away from you, and gently slide it into the clamp assembly along the front face of the acrylic pressure plate. The longer glass plate should be against the acrylic pressure plate of the clamp assembly. Tighten the top two screws of the clamp assembly.

**Note:** The clamp assembly has six screws. The top and bottom screws, turned by hand, hold the gel sandwich in place. The middle screw holds the clamp assembly together. It requires a screwdriver to move it and should not be adjusted.

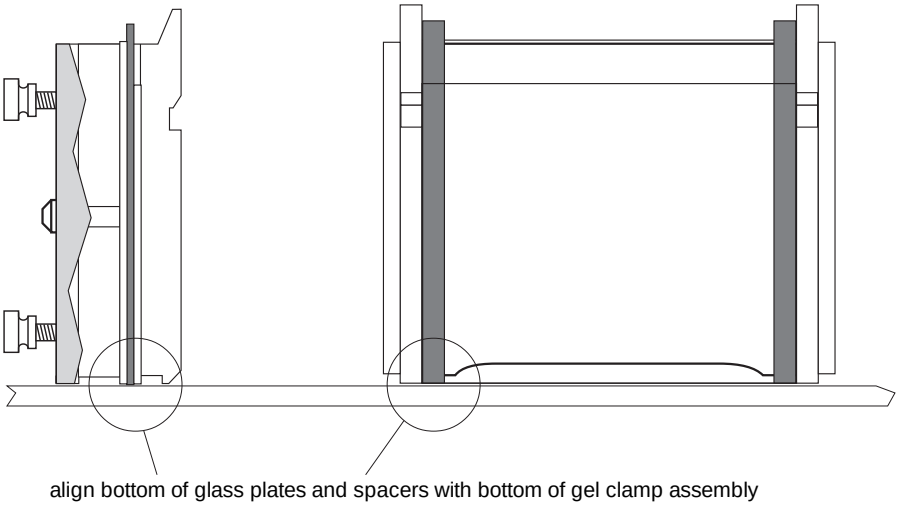



**Fig. 3.2**

3. Place the clamp assembly into the alignment slot so that the clamp screws face away from you (Figure 3.3). Loosen the top two screws to allow the plates and spacers to settle against the casting stand base. Insert the Mini-PROTEAN II alignment card between the glass plates, in order to position the spacers properly. Gently tighten both pairs of screws (Figure 3.4).
4. Remove the alignment card. Pull the completed sandwich from the alignment slot. Check that the plates and spacers are flush at the bottom. If not, realign the sandwich as in steps 1-3.
5. Using the leveling bubble, level the casting stand with the alignment slot facing you. Check to see that the removable gray silicone gaskets are clean and free of residual acrylamide to insure a good seal. Place the silicone rubber gaskets on top of the red foam pads of the casting stand slots.

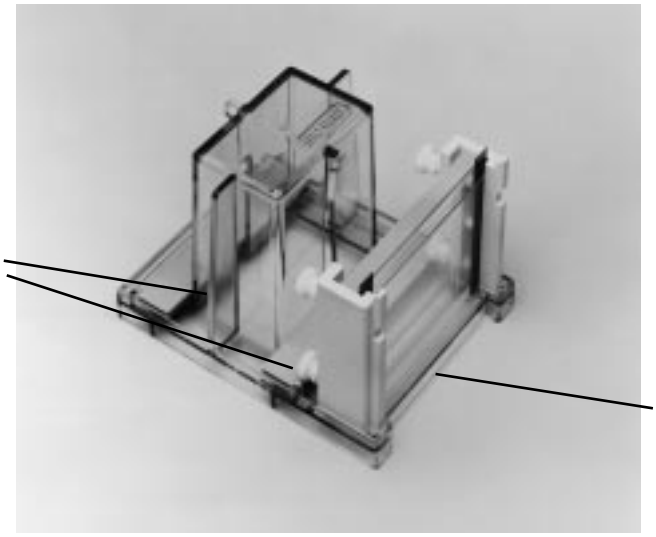



**Fig. 3.3**

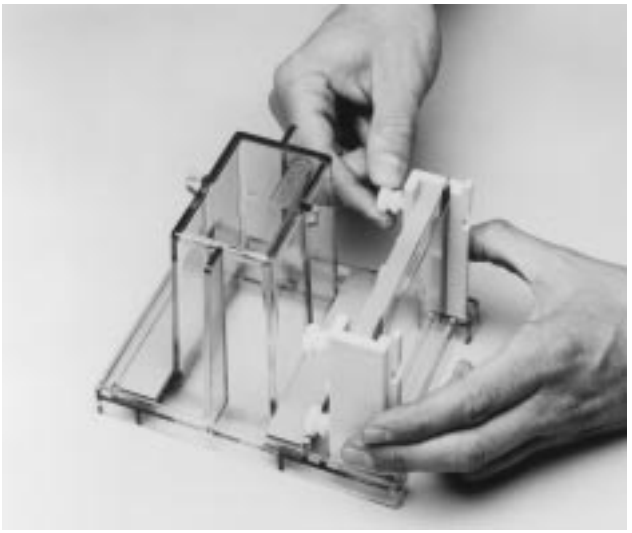


**Fig. 3.4**

**Note:** Before transferring the clamp assembly to the casting slot, recheck the alignment of the spacers. Do this by inverting the gel sandwich and looking at the surface of the two glass plates and the spacer. **All three surfaces must be flush.** If a spacer is crooked, or one of the glass plates is higher than the other, the gel sandwich could leak while the gel is being poured, or the upper buffer could leak from the misaligned plates during the electrophoresis run. If the surfaces of all three components are not flush, repeat the alignment procedure. Do not proceed until the gel sandwich is properly aligned in the clamp assembly.

6. Transfer the clamp assembly to one of the casting slots in the casting stand. If two gels are to be cast, place the clamp assembly on the side opposite the alignment slot to make aligning the next sandwich easier.
7. Attach the sandwich in the following way: Butt the acrylic pressure plate against the wall of the casting slot at the bottom, so the glass plates rest on the rubber gasket (see Figure 3.5). Snap the acrylic plate underneath the overhang of the casting slot by pushing with the white portions of the clamps (see Figure 3.6). Do not push against the glass plates or spacers. This could break the plate.

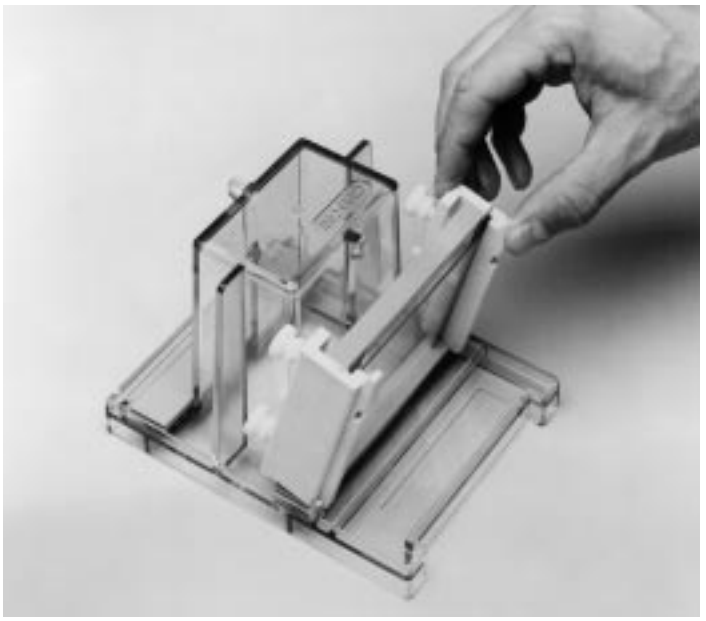


**Fig 3.5**

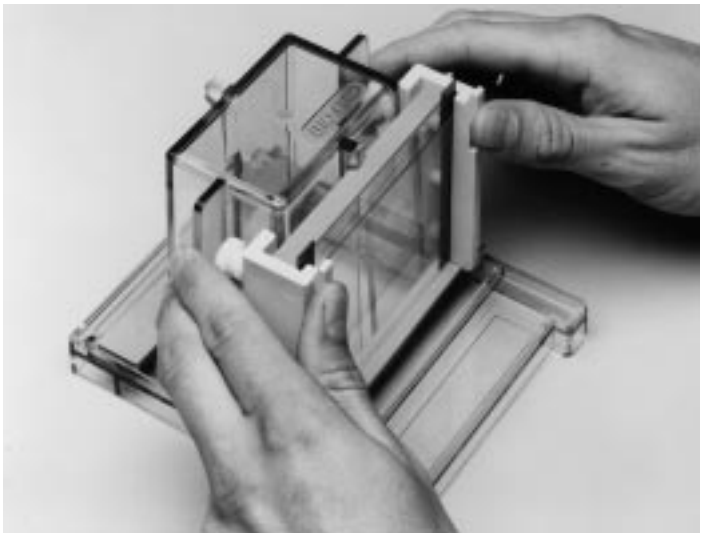


**Fig 3.6**

8. If another gel is to be cast, align the sandwich plates in the clamp assembly, and then transfer to the other casting slot of the casting stand. The gel sandwiches are now ready for casting.

# Section 4
# Casting the Gels

## 4.1 Casting Discontinuous (Laemmli) Polyacrylamide Gels

Discontinuous polyacrylamide gels consist of resolving or separating (lower) gels and stacking (upper) gels. The stacking gels act to concentrate large sample volumes, resulting in better band resolution than is possible by using the same volumes on a gel without a stack. Molecules are then separated in the resolving gel. The most popular discontinuous buffer system is that of Laemmli.* The formulations for this system are included in the appendix. For a complete guide to the process of acrylamide polymerization, request bulletin 1156, "Acrylamide Polymerization-A Practical Approach."

1. Prepare the separating gel monomer solution by combining all reagents except ammonium persulfate (APS) and TEMED (see Section 11.2 for formulations). Degas the solution under vacuum for at least 15 minutes. Water aspirators on the sink generally do not provide an adequate vacuum for complete degassing.

2. Place a comb completely into the assembled gel sandwich. With a marker pen, place a mark on the glass plate 1 cm below the teeth of the comb. This will be the level to which the separating gel is poured. Remove the comb.

3. Add APS and TEMED to the degassed monomer solution and pour the solution to the mark, using a glass pipet and bulb or disposable plastic transfer pipet (DPTP). The easiest way to pour is to flow the solution down the middle of the longer plate of the gel sandwich. Another way to pour is to flow the solution down the side of one of the spacers. Pour the solution smoothly to prevent it from mixing with air.

4. Immediately overlay the monomer solution with water, water-saturated isobutanol, or t-amyl alcohol. The advantage of using isobutanol or t-amyl alcohol is that the overlay solution can be applied rapidly with a Pasteur pipet and bulb because very little mixing will occur. If water is used to overlay, it must be done more slowly, with a steady, even rate of delivery to prevent mixing.

   **Note:** Isobutanol or t-amyl alcohol will chemically attack the acrylic plastic of the sandwich clamp. This will cause crazing of the plastic. This crazing renders the plastic opaque, which makes sample application difficult. If alcohol is used to overlay the monomer solution, exercise caution to avoid contact with the acrylic plastic.

5. Allow the gel to polymerize for 45 minutes to 1 hour. Rinse off the overlay solution completely with distilled water. This is especially important with alcohol overlays. Do not allow alcohols to remain on the gels more than 1 hour, or the top of the gel will dehydrate.

   **Note:** It is sometimes convenient to cast the separating portion of a discontinuous gel the afternoon before casting the stacking gel and running the gel. If the stacking gel is to be cast the following day, place approximately 5 ml of 1:4 solution B (see Section 11.1) on top of each separating gel after rinsing with deionized water. This will prevent dehydration of the separating gel during overnight storage at room temperature.

6. Prepare the stacking gel monomer solution. Combine all reagents except APS and TEMED and degas under vacuum at least 15 minutes.

7. Dry the area above the separating gel with filter paper before pouring the stacking gel.

*Laemmli, U. K., Nature, 227, 680 (1970)

8. Place a comb in the gel sandwich and tilt it so that the teeth are at a slight (~10°) angle. This will prevent air from being trapped under the comb teeth while the monomer solutions are poured.

9. Add APS and TEMED to the degassed monomer solution and pour the solution down the spacer nearest the upturned side of the comb. Pour until all the teeth have been covered by solution. Then properly align the comb in the sandwich and add monomer to fill completely. The comb is properly seated when the T portion of the comb rests on top of the spacers.

10. Allow the gel to polymerize 30-45 minutes. Remove the comb by pulling it straight up slowly and gently.

11. Rinse the wells completely with distilled water or running buffer. The gels are now ready to be attached to the inner cooling core, the sample loaded, and the gels run.

## 4.2 Casting Continuous Gels

Continuous gels are ones in which the entire gel is of one composition. This type of gel is often used in non-denaturing (native) buffer systems. See Section 13.2 for references to the most commonly used native buffer systems.

1. Prepare the monomer solution. Combine all reagents except APS and TEMED. Degas under vacuum for at least 15 minutes.

2. Place a comb in the glass sandwich so that the teeth are tilted at approximately a 10° angle.

3. Add APS and TEMED to the degassed monomer solution and use a pipet and bulb to pour the solution down the spacer nearest to the upturned side of the comb. Pour until the bottoms of all the teeth are covered. Then adjust the comb to its proper position. Add monomer solution to fill the sandwich completely.

4. Let the gel polymerize for 45 minutes to 1 hour. The gel is now ready to load and run. Remove the comb as in Section 4.1, and rinse the wells thoroughly with distilled water.

# Section 5
# Assembling the Upper Buffer Chamber

## 5.1 Assembly

**Note:** To insure a leakproof seal, make sure the gray U-shaped inner cooling core gaskets are clean. Inspect the gasket for small cuts that could result in an upper buffer leak. There are two sides to this gasket. Make sure that the side with the notch is exposed for contact with the gel sandwich.

1. Release the clamp assemblies/gel sandwiches from the casting stand.
2. Lay the inner cooling core down flat on a lab bench. With the glass plates of the gel sandwich facing the cooling core (and the clamp screws facing out), carefully slide the clamp assembly wedges underneath the locator slots on the inner cooling core until the inner glass plate of the gel sandwich butts up against the notch in the U-shaped gasket (Figure 5.1).

   **Note:** Lubricating the raised portions of the U-shaped gasket with a drop of running buffer or water helps the glass plate sandwich slide in properly.

   While pushing the clamp assembly slightly up toward the top of the locator slots, snap the clamp assembly fully onto the cooling core by pressing at the bottom of the clamp assembly until the cooling core latch engages each side of the clamp assembly. (Do not pull out on cooling core latch at the same time.)

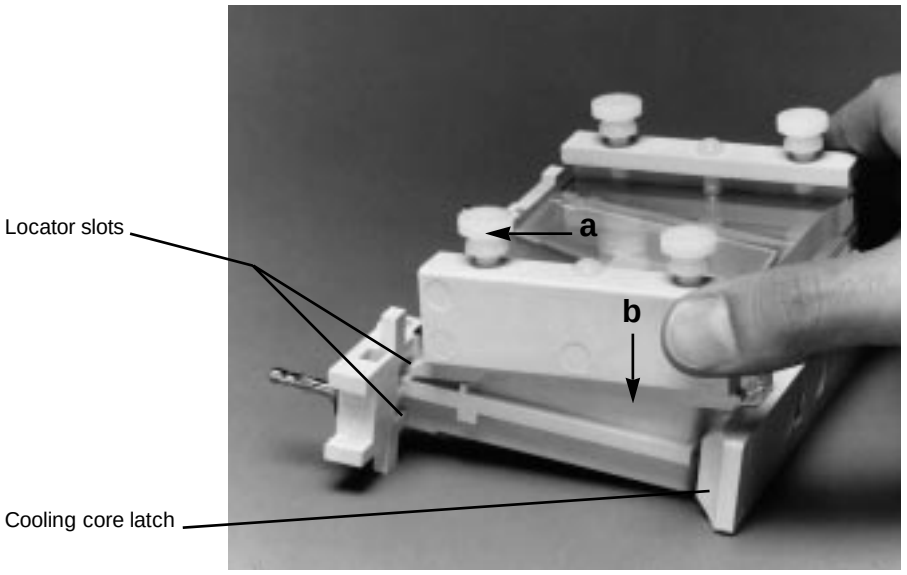
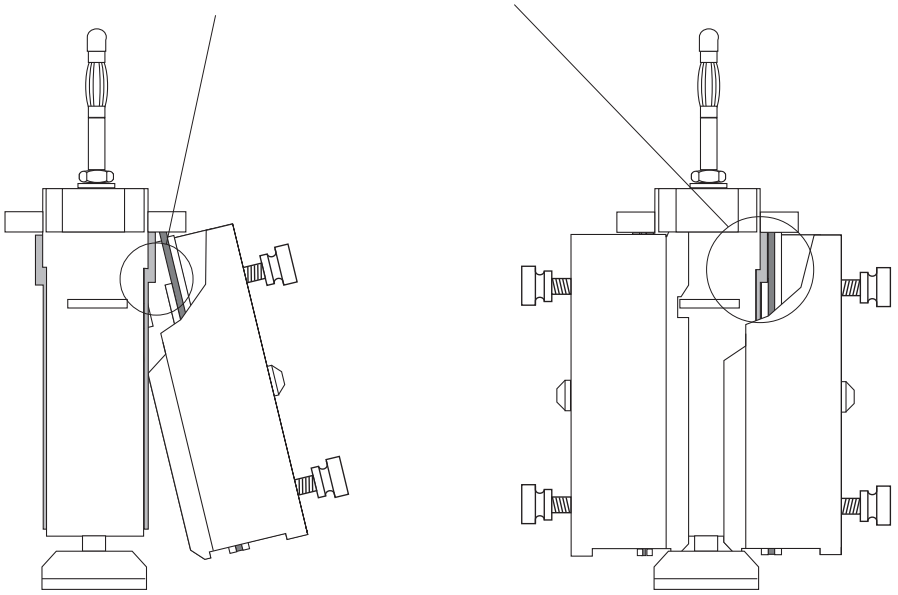



**Fig 5.1**

**Note:** It is very important that there is good contact at the point where the short glass plate contacts the notch in the gasket to prevent upper buffer leaks (see Figure 5.2). If the glass plate is not firmly seated in its proper place against the notch in the gasket, buffer leaks will occur.



(correct alignment of plate and gasket)

**Fig 5.2**

3. Turn over the inner cooling core and attach another clamp assembly to the other side of the core in the same manner.

## 5.2 Using a Buffer Dam (Running a Single Gel)

If only one gel is to be run, a buffer dam may be made by sliding a short (inner) glass plate and a long (outer) glass plate *without spacers* into the clamp assembly as in Section 3. Align the plates using the alignment slot of the casting stand, tighten the clamp screws, and snap onto the inner cooling core.

# Section 6
# Loading The Samples

Sample loading can be done in two ways. The most common method is to load samples into wells formed in the gel with a well-forming comb. The second method uses the entire gel surface as a single well for liquid samples.

## 6.1 Loading Sample Wells

The approximate maximum sample volume that each well will hold is given in Section 12.3.

1. Prepare 300 ml of electrode buffer by combining 60 ml of 5X electrode buffer (see Section 11.1) with 240 ml of deionized water.

2. Lower the inner cooling core into the lower buffer chamber of the Mini-PROTEAN II cell. Add approximately 115 ml of buffer to the upper buffer chamber. Fill until the buffer reaches a level halfway between the short and long plates. Do not overfill the upper buffer chamber.

   **Note:** Overfilling the upper buffer chamber will result in siphoning of the buffer over the top of the gasket, resulting in buffer loss and interruption of the electrophoresis experiment.

3. Pour the remainder of the buffer into the lower buffer chamber so that at least the bottom 1 cm of the gel is covered. Remove any air bubbles from the bottom of the gel so that good electrical contact is achieved. This can be done by swirling the lower buffer with a pipet until the bubbles clear.

4. Load the samples into the wells under the electrode buffer with a Hamilton syringe or with a pipettor using Bio-Rad Prot/Elec tips (catalog number 223-9917). Insert the syringe to about 1-2 mm from the well bottom before delivery. Disposable pieces of plastic tubing may be attached to the syringe to eliminate the need for rinsing the syringe between samples.

   **Note:** The sample buffer must contain either 10% sucrose or 10% glycerol in order to underlay the sample in the well without mixing.

## 6.2 Loading a Single Sample Per Gel

In this procedure, a gel is cast without using a comb, forming a flat gel surface. This gel is cast with an overlay solution. This type of sample application can be used for preparative purposes, but it is most often used in blotting applications. Follow the instructions for casting the separating portions of a discontinuous gel (Section 4.1). Pour the stacking gel to within 1 mm of the top of the short plate. Overlay with overlay solution. After polymerization, rinse as usual. Assemble the core.

1. Prepare electrode buffer and add to upper and lower reservoirs as in Section 6.1.

2. The sample may be loaded with a pipettor, with a needle and syringe, or Hamilton syringe. Start at one end of the gel and deliver the sample gently and evenly over the entire length of the gel. Layer the sample as close as possible (1-2 mm) to the surface of the gel.

## Section 7
## Running The Gel

1. Place the lid on top of the lower buffer chamber to fully enclose the cell. The correct orientation is made by matching the colors of the plugs on the lid with the jacks on the inner cooling core.
2. Attach the electrical leads to a suitable power supply (200 V minimum) with the proper polarity.
3. Apply power to the Mini-PROTEAN II cell and begin electrophoresis. The recommended power condition for optimal resolution with minimal thermal band distortion is 200 volts, constant voltage setting. No adjustment of the setting is necessary for spacer thickness or number of gels. The usual run time is approximately 45 minutes. Current should be approximately 60 mA per gel (120 mA for two gels) at the beginning of the run. During the 45 minute run the current will slowly drop to about 30 mA per gel. This drop is caused by the change in buffer ions in the gel, causing a slow rise in the resistance in the gel. As one would expect from Ohms law (V=I*R), at constant voltage (V) a rise in the resistance (R) results in a drop in the current (I).

   Bio-Rad's power supplies which regulate in constant voltage mode are listed in Section 12.6.

## Section 8
## Removing The Gel

1. After electrophoresis is complete, turn off the power supply and disconnect the electrical leads.
2. Remove the cell lid and carefully pull the inner cooling core out of the lower chamber. Pour off the upper buffer.
3. Lay the inner cooling core on its side and remove the clamp assembly by pushing down on both sides of the cooling core latch and up on the clamps until the clamp assembly is released (Figure 8.1). Slide the clamp assembly away from the cooling core.

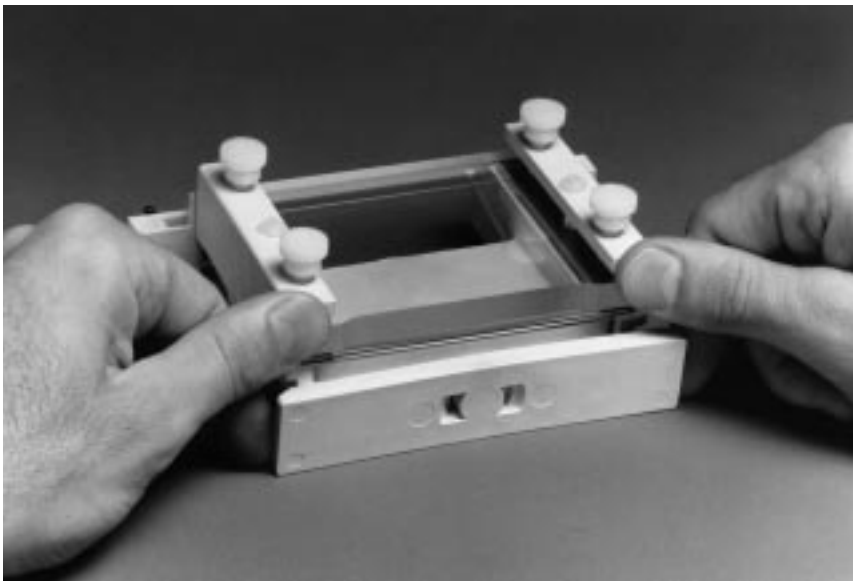


**Fig 8.1**

### Removing The Gel (Continued)

4. Loosen all four screws of the clamp assembly and remove the glass plate sandwich from it.
5. Push one of the spacers of the sandwich out to the side of the plates without removing it.
6. Gently twist the spacer so that the upper glass plate pulls away from the gel. Remove the plate. The gel will stick to one of the plates.
7. Float the gel off of the glass plate by inverting the gel and glass plate under fixative solution or blotting transfer buffer and agitate gently until the gel separates from the glass plate. (See Section 11.5 for fixing and staining protocols.)

## Section 9
## Maintenance of Equipment

| | |
|---|---|
| Mini-PROTEAN II cell chamber, inner core, clamps: | Rinse thoroughly with distilled water after every use. |
| Glass plates, spacers, combs: | Wash with a laboratory detergent (catalog number 161-0722), then rinse thoroughly with distilled water. |
| Glass plates (when more stringent cleaning is required): | Soak in a strong acid solution (chromic acid/sulfuric acid cleaning solution) for ≥ 30 minutes and then rinse thoroughly with distilled water. |

Warning: Exercise extreme caution for acid cleaning; wear safety glasses, a lab coat, and rubber gloves. Keep a container of $NaCO_3$ nearby to neutralize spills.

# Section 10
# Troubleshooting Guide-PAGE, SDS-PAGE

| | Problem | | Cause | | Solution |
|---|---|---|---|---|---|
| 1. | "Smile effect" - band pattern curves upward at both sides of the gel. | a. | Center of the gel running hotter than either end. | a. | Decrease power setting, or fill lower chamber to within 1 cm of top of short glass plate. |
| | | b. | Power conditions excessive. | | |
| 2. | Diffuse tracking dye. | a. | Decomposition of sample solution and/or buffer stock solutions. | a. | Prepare fresh reagents. Maximum shelf life of aqueous solutions is 30 days at 4° C for buffer and monomer stocks. |
| | | b. | BPB concentration too high. | b. | Remake sample buffer w/BPB concentration at 0.025%. |
| 3. | Vertical streaking of protein. | a. | Sample overload. | a. | Dilute sample, selectively remove predominant protein in the sample, or reduce voltage by about 25% to minimize streaking. |
| | | b. | Sample precipitation. | b. | Centrifuge sample before addition of SDS sample buffers, or decrease % T of resolving gel.* |
| | | | | c. | The ratio of SDS to protein should be enough to coat each protein molecule with SDS, generally 1.4:1. It may require more SDS for some membrane protein samples. |
| 4. | Lateral band spreading. | a. | Diffusion out of the wells prior to turning on the current | a. | Minimize the time between sample application and power start up. |
| | | b. | Diffusion during migration through the stacking gel. | b. | Increase % T of stacking gel to 4.5% or 5% T, or increase current by 25% during stacking.* |
| | | c. | Ionic strength of sample lower than that of gel. | c. | Match ionic strength of sample buffer to that of the gel. |
| 5. | Skewed or distorted bands. | a. | Poor polymerization around sample wells. | a. | Degas stacking gel solution thoroughly prior to casting; increase ammonium persulfate and TEMED concentrations by 25%, also wipe comb teeth with Temed just before casting the stacking gel. |
| | | b. | Salts in sample. | b. | Remove salts by dialysis, desalting column, etc. |
| | | c. | Uneven gel interface. | c. | Decrease the polymerization rate. Overlay gels very carefully. |
| 6. | Lanes constricted at bottom of gel. | a. | Ionic strength of sample higher than that of surrounding gel. | a. | Desalt sample and neighboring samples. |

| | Problem | Cause | Solution |
|---|---|---|---|
| 7. | Run taking unusually long time. | a. Running buffer too concentrated. | a. Check buffer protocol, dilute if necessary. |
| | | b. Low current. | b. Increase voltage by 25-50%. |
| | | c. Excessive salt in sample. | |
| 8. | Run too fast, poor resolution. | a. Running or reservoir buffer too dilute. | a. Check buffer protocol, concentrate if necessary. |
| | | b. Voltage too high. | b. Decrease voltage by 25-50%. |
| 9. | Doublets observed where a single protein species is expected (SDS-PAGE) | a. A portion of the protein may have been re-oxidized during the run or may not have been fully reduced prior to run. | a. Prepare fresh sample buffer solutions if over 30 days old; increase 2-mercaptoethanol concentration in the sample buffer. |
| 10. | Observe fewer bands than expected and one heavy band at dye front. | a. More than one band migrating at the dye front. | a. Increase % T of resolving gel.* |
| | | b. Protein degradation. | |
| 11. | Upper buffer chamber leaks. | a. Upper buffer chamber over filled. | a Keep level of buffer below the top of the long glass plates. |
| | | b. Improper assembly . | b. Be sure core gasket is clean, free of cuts, and lubricated with buffer. Be sure short glass plate is *under* the notch on the gasket, not on top of it. |
| 12. | Leaking during gel casting. | a. Improper assembly of gel sandwich. | a. Use the alignment card and check to be sure the spacers and glass plate bottoms are flush prior to pouring gel. |
| | | b. Chipped glass plates. | b. Insure glass plates are free of flaws. |
| 13. | Clamp assembly will not fit into casting stand. | a. Incorrectly assembled. | a. Make sure grey and red gaskets are in place. |
| | | | b. Check acrylic block. It must be in the correct orientation, with the curved part at the bottom of the clamps. |
| | | | c. Screws should be facing away from the clamp support. |

*Polyacrylamide gels are described by reference to two characteristics:

1) The total monomer concentration, (%T) and

2) The crosslinking monomer concentration (%C).

$$\%T = \frac{\text{g acrylamide + g bis-acrylamide}}{\text{Total Volume}} \times 100$$

$$\%C + \frac{\text{g bis- acrylamide}}{\text{g acrylamide + g bis-acrylamide}} \times 100$$

# Section 11
# Appendix

## 11.1 Reagents and Gel Preparation for SDS-PAGE Slab Gel (Laemmli buffer system*)

### Stock Solutions

A. Acrylamide/bis (30% T, 2.67% C)

87.6 g acrylamide (29.2g/100 ml)

2.4 g N'N'-bis-methylene-acrylamide (0.8 g/100 ml)

Make to 300 ml with deionized water. Filter and store at 4 °C in the dark (30 days maximum).

Or, use

Bio-Rad's Preweighed Acrylamide/Bis 37.5:1 mixture
(catalog number 161-0112, 30 g)
(catalog number 161-0106, 200 g))

Bio-Rad's 30% Acrylamide/BIS solutions 37.5:1 mixture
(catalog number 161-0158, 500 ml)
(catalog number 161-0159, 2 x 500 ml)

B. 1.5 M Tris-HCl, pH 8.8

27.23 g Tris base (18.15 g/100 ml)

~80 ml deionized water

Adjust to pH 8.8 with 6N HCl. Make to 150 ml with deionized water and store at 4 °C.

C. 0.5 M Tris-HCl, pH 6.8

6 g Tris base

~60 ml deionized water

Adjust to pH 6.8 with 6N HCl. Make to 100 ml with deionized water and store at 4 °C.

D. 10% SDS

Dissolve 10 g SDS in 90 ml water with gentle stirring and bring to 100 ml with $ddH_2O$.

E. Sample buffer (SDS reducing buffer) (store at room temperature)

| | |
|---|---|
| Deionized water | 3.8 ml |
| 0.5 M Tris-HCl, pH 6.8 | 1.0 ml |
| Glycerol | 0.8 ml |
| 10% (w/v) SDS | 1.6 ml |
| 2-mercaptoethanol | 0.4 ml |
| 1% (w/v) bromophenol blue | 0.4 ml |
| | 8.0 ml |

Dilute the sample at least 1:4 with sample buffer, and heat at 95 °C for 4 minutes.

F. 5X electrode (Running) buffer, pH 8.3 (enough for 10 runs)

| | | |
|---|---|---|
| Tris base | 9 g | (15 g/l) |
| Glycine | 43.2 g | (72 g/l) |
| SDS | 3 g | (5 g/l) |

to 600 ml with deionized water

Store at 4 °C. Warm to room temperature before use if precipitation occurs.

Dilute 60 ml 5X stock with 240 ml deionized water for one electrophoretic run.

*Laemmli, U. K.,Nature, 227, 680 (1970)

## 11.2 Separating Gel Preparation - 0.375 M Tris, pH 8.8

| | 12%[a] | 7.5%[b] |
|---|---|---|
| Deionized water | 3.35 ml | 4.85 ml |
| 1.5M Tris-HCI< pH 8.8 | 2.5 ml | 2.5 ml |
| 10% (w/v) SDS stock | 100 µl | 100 µl |
| (store at room temperature) | | |
| Acrylamide/Bis (30% stock) (Degas for ≥ 15 minutes at room temperature) | 4.0 ml | 2.5 ml |
| *10% ammonium persulfate (fresh daily) | 50 µl | 50 µl (0.05%) |
| TEMED | 5 µl | 5 µl (0.05%) |
| TOTAL MONOMER+ | 10 ml | 10 ml |

*To make the 10% ammonium persulfate solution, dissolve 100 mg APS in 1 ml deionized water.
+One can prepare any desired volume of monomer solution by using multiples of the 10 ml recipes.

a. For SDS treated proteins in the approximate molecular weight range of 10-100 k daltons. Use Bio-Rad's Low MW Standards (catalog number 161-0304) for 12% separating gel.

b. For SDS treated proteins in the approximate molecular weight range of 40-250 k daltons. Use in conjunction with Bio-Rad's High MW SDS-PAGE Standards (catalog number 161-0303.)

### Calculated Volumes* (in ml) Required Per Gel Slab

| Spacer Thickness | Volume |
|---|---|
| 0.5 mm | 2.8 ml |
| 0.75 mm | 4.2 ml |
| 1.0 mm | 5.6 ml |
| 1.5 mm | 8.4 ml |

*Volume required to completely fill gel sandwich. Amounts may be adjusted depending on application (with or without comb, with or without stacking gel, etc.).

## 11.3 Stacking Gel Preparation - 4.0% gel, 0.125 M Tris, pH 6.8

| | | |
|---|---|---|
| Deionized water | 6.1 ml | |
| 0.5 M Tris-HCI, pH 6.8 | 2.5 ml | |
| 10% (w/v) SDS | 100 µl | |
| Acrylamide/bis (30% stock) | 1.33 ml | |
| 10% ammonium persulfate (fresh daily) | 50 µl | (.05%) |
| TEMED | 10 µl | (0.1%) |
| TOTAL STOCK MONOMER | 10 ml[a] | |

[a] Enough for two gels, all thicknesses.

1. To prepare the monomer solutions, combine all reagents, except the TEMED and APS, and degas under vacuum for ≥ 15 minutes. To initiate polymerization add the APS and TEMED, and swirl gently to mix.

2. Follow the instructions in Sections 3 and 4 for set-up and casting the gels.

## 11.4 Running Conditions

The recommended power condition for optimal resolution with minimal thermal band distortion is 200 volts, constant voltage setting. No adjustment of the setting is necessary for spacer thickness or number of gels. The usual run time is approximately 45 minutes.

## 11.5 Comparison of Coomassie Blue and Silver Staining

| | **Coomassie Blue** | **Sliver Stain** |
|---|---|---|
| Procedure | Stain 1/2 hour with 0.1% Coomassie blue R-250 in fixative (40% MeOH, 10% HOAc)<br><br>Destain with several changes of 40% MeOH/ 10% HOAc to remove background (usually 1 to 3 hr). | As per instructions in kit. 1 hour and 45 minutes for 0.75 mm gel. |
| Sensitivity | µg range | ng range |

# Section 12
# Equipment and Accessories

## 12.1 Mini-PROTEAN II Slab Cell

| Catalog Number | Product Description |
|---|---|
| 165-2940 | **Mini-PROTEAN II Cell,** 10 well combs, 0.75 mm spacers, includes inner cooling core with gaskets, lower buffer chamber, lid with power cables, glass plates (3 sets), 2 clamp assemblies, 10 well, 0.75 thick combs (2), 0.75 mm thick spacers 4), casting stand with gasket, leveling bubble, and instructions. |
| 165-2941 | **Mini-PROTEAN II Cell,** same as above without combs and spacers. |
| 165-2941 | **Mini-PROTEAN II Module,** same as 165-2940 without tank and lid. |

## 12.2 Accessories

| | |
|---|---|
| 165-2943 | **Casting Stand with Gaske**t, to cast 1 or 2 gels |
| 165-2904 | **Replacement Gaskets**, casting stand, 2 |
| 165-2946 | **Clamp Assembly**, to cast 1 gel |
| 165-2902 | **Replacement Red Foam Gaskets,** casting stand, 2 |
| 165-2905 | **Replacement Gasket**, inner cooling core, 2 |
| 165-2912 | **Glass Plates**, 10 sets* |
| 165-2907 | **Inner Glass Plates**, 10 |
| 165-2908 | **Glass Plates**, 10 |
| 165-2975 | **Buffer Tank and Lid** |
| 165-2942 | **Mini-PROTEAN II Electrode Assembly,** with gaskets |
| 165-2948 | **Replacement Power Cables**, 1 pair IEC 1010-1 approved |

*One set = one short (inner) plate and one long (outer) plate (enough to cast one gel).

| Catalog Number | Product Description |
|---|---|
| **Teflon® Combs** | |
| 165-2915 | **5 well x 0.5 mm** |
| 165-2916 | **5 well x 0.75 mm** |
| 165-2917 | **5 well x 1.0 mm** |
| 165-2918 | **5 well x 1.5 mm** |
| 165-2919 | **10 well x 0.5 mm** |
| 165-2920 | **10 well x 0.75 mm** |
| 165-2921 | **10 well x 1.0 mm** |
| 165-2922 | **10 well x 1.5 mm** |
| 165-2936 | **9 well x 0.5 mm** |
| 165-2937 | **9 well x 0.75 mm** |
| 165-2938 | **9 well x 1.0 mm** |
| 165-2939 | **9 well x 1.5 mm** |

| Catalog Number | Product Description |
|---|---|
| **Teflon Combs *(continued)*** | |
| 165-2923 | **15 well x 0.5 mm** |
| 165-2924 | **15 well x 0.75 mm** |
| 165-2925 | **15 well x 1.0 mm** |
| 165-2926 | **15 well x 1.5 mm** |
| 165-2927 | **Preparative x 0.75 mm, 1 reference well** |
| 165-2928 | **Preparative x 1.0 mm, 1 reference well** |
| 165-2929 | **Preparative x 1.5 mm, 1 reference well** |
| **Spacer Sets** | |
| 165-2930 | **0.5 mm, 4** |
| 165-2931 | **0.75 mm, 4** |
| 165-2932 | **1.0 mm, 4** |
| 165-2933 | **1.5 mm, 4** |

## 12.3 Mini-PROTEAN II Multi-Casting Chamber and Accessories

| Catalog Number | Product Description |
|---|---|
| 165-2950 | **Mini-PROTEAN II Multi-Casting Chamber**, includes casting chamber body with front captive screws, cover with gasket, 10 sets of glass plates, 15 separation sheets, set of 4 acrylic blocks, stopcock valve, luer fittings, and instructions. |
| 165-2913 | **Replacement Gaskets**, Mini-PROTEAN II multi-casting chamber, 3 |
| 165-2956 | **Separation Sheets**, 15 sheets |
| 165-2912 | **Glass Plates**, 10 sets |
| 165-2955 | **Acrylic Blocks**, set of 4 |

## 12.4 Modular Mini Components for 2-D, Blotting, and Electro-Elution

| Catalog Number | Product Description |
|---|---|
| 170-3935 | **Mini Trans-Blot Module**, for use with the Mini-PROTEAN II cell, includes gel holder cassettes (2), fiber pads (4), modular electrode assembly, Bio-Ice® cooling unit, and instruction manual. |
| 165-2960 | **Mini-PROTEAN II 2-D Cell**, includes tube cell module, sample reservoir and stoppers, sample reservoir/capillary tube connectors (50), capillary tubes (200) with casting tube, tube gel ejector, Mini-PROTEAN II slab cell with inner cooling core and gaskets, lower buffer chamber, lid with power cables, glass plates (3 sets),* clamp assemblies (2), 2-D combs with 1 standard well (2), 1.0 mm thick spacers (4), casting stand with gaskets, leveling bubble, and 2-D instruction manual. |

| Catalog Number | Product Description |
|---|---|
| 165-2965 | **Mini-PROTEAN II Tube Cell Module**, for use with the Mini-PROTEAN II cell, includes tube cell module, sample reservoirs and stoppers, sample reservoir/capillary tube connectors (50), capillary tubes (200) with casting tube, tube gel ejector, and instruction manual. |
| 165-2976 | **Model 422 Electro-Eluter**, includes electro-eluter module, clear Membrane Caps (12), glass tubes with frits (6), silicone adaptors (6), grommets and stoppers (6), buffer tank with lid and cables, and instruction manual. |
| 165-2977 | **Model 422 Electro-Eluter Module**, same as 165-2976 without buffer tank and lid. |

## 12.5 Power Supplies

| Catalog Number | Product Description |
|---|---|
| 165-5056 | **PowerPac 3000 Power Supply**, 100/120 V |
| 165-5057 | **PowerPac 3000 Power Supply**, 200/240 V |
| 165-4710 | **Model 1000/500 Power Supply**, 100/120 V |
| 165-4711 | **Model 1000/500 Power Supply**, 220/240 V |
| 165-4761 | **Model 200/2.0 Constant Voltage Power Supply**, 100/120V 50/60 Hz |
| 165-4762 | **Model 200/2.0 Constant Voltage Power Supply**, 220/240V 50/60 Hz |
| 165-5050 | **PowerPac 300 Power Supply,** 100/120 V |
| 165-5051 | **PowerPac 300 Power Supply,** 220/240 V |

## 12.6 Electrophoresis Chemicals

### Precast Gels for the Mini-PROTEAN II cell

| Catalog Number | Product Description |
|---|---|
| **Tris-HCl Gels for PAGE and SDS-PAGE** | |
| 161-0900 | **Ready Gel,** 7.5% resolving gel, 4% stacking gel |
| 161-0901 | **Ready Gel,** 12% resolving gel, 4% stacking gel |
| 161-0902 | **Ready Gel,** 4-15% gradient gels |
| 161-0903 | **Ready Gel,** 4-20% gradient gels |
| 161-0906 | **Ready Gel,** 10-20% gradient gel, 4% stacking gel |
| 161-0907 | **Ready Gel,** 10% resolving gel, 4% stacking gel |
| 161-0908 | **Ready Gel,** 15% resolving gel, 4% stacking gel |
| 161-0909 | **Ready Gel,** same as 161-0901 with 2 well prep comb |
| **Tris-Borate-EDTA Gels for DNA** | |
| 161-0904 | **Ready Gel,** 5% TBE gels |
| 161-0905 | **Ready Gel,** 10% TBE gels |
| **Tris-Tricine Gels for Peptides and Small Proteins** | |
| 161-0922 | **Ready Gel,** 16.5% resolving gel, 4% stacking gel |
| 161-0923 | **Ready Gel,** 10-20% gradient gel, 4% stacking gel |

## Acrylamide/Bis Premixed Powders and Solutions

| Catalog Number | Product Description | Quantity per Package |
|---|---|---|
| 161-0122 | **Preweighed Acrylamide/Bis**, 37.5:1 mixture | 30 g |
| 161-0125 | **Preweighed Acrylamide/Bis**, 37.5:1 mixture | 150 g |
| 161-0121 | **Preweighed Acrylamide/Bis**, 29:1 mixture | 30 g |
| 161-0124 | **Preweighed Acrylamide/Bis**, 29:1 mixture | 150 g |
| 161-0154 | **30% Acrylamide/Bis Solution,** 19:1 | 500 ml |
| 161-0155 | **30% Acrylamide/Bis Solution,** 19:1 | 2 x 500 ml |
| 161-0156 | **30% Acrylamide/Bis Solution,** 29:1 | 500 ml |
| 161-0157 | **30% Acrylamide/Bis Solution,** 29:1 | 2 x 500 ml |
| 161-0158 | **30% Acrylamide/Bis Solution,** 37.5:1 | 500 ml |
| 161-0159 | **30% Acrylamide/Bis Solution,** 37.5:1 | 2 x 500 ml |

## Premixed Buffers

| Catalog Number | Product Description | Quantity per Package |
|---|---|---|
| 161-0732 | **10x Tris/Glycine/SDS** | 1 L |
| 161-0755 | **10x Tris/Glycine/SDS** | 6 x 1 L |
| 161-0734 | **10x Tris/Glycine** | 1 L |
| 161-0757 | **10x Tris/Glycine** | 6 x 1 L |
| 161-0733 | **10x Tris/Boric acid/EDTA** | 1 L |
| 161-0756 | **10x Tris/Boric acid/EDTA** | 6 x 1 L |
| 161-0741 | **10x TBE Extended Range** | 1 L |
| 161-0758 | **10x TBE Extended Range** | 6 x 1 L |

| Catalog Number | Product Description | Quantity per Package |
|---|---|---|
| 161-0416 | **10% SDS Solution** | 250 ml |
| 161-0418 | **20% SDS Solution** | 1000 ml |
| 161-0700 | **Ammonium Persulfate** | 10 g |
| 161-0800 | **TEMED** | 5 ml |
| 161-0801 | **TEMED** | 50 ml |
| 161-0710 | **2-mercaptoethanol** | 25 ml |
| 161-0611 | **Dithiothreitol** | 5 g |
| 161-0404 | **Bromophenol Blue** | 10 g |

## Electrophoresis Stains

| Catalog Number | Product Description | Quantity per Package |
|---|---|---|
| 161-0400 | **Coomassie Blue R-250** | 10 g |
| 161-0406 | **Coomassie Blue G-250** | 10 g |
| 161-0443 | **Silver Stain Kit** | |
| 161-0449 | **Silver Stain Plus Kit** | |
| 161-0433 | **Ethidium Bromide Solution,** 10 mg/ml | 10 ml |

# Section 13
# References

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