



Mini-PROTEAN® II
Electrophoresis Cell
Instruction Manual



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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 core, 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 (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

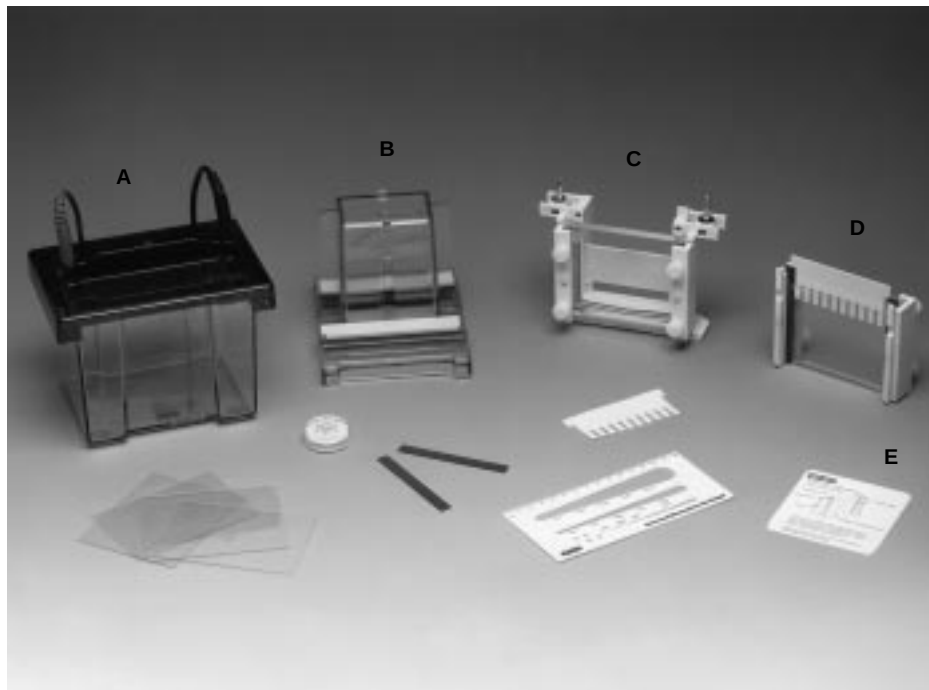


Fig. 2.1 Mini-PROTEAN II electrophoresis cell, lower buffer chamber, and lid (a), casting stand (b), inner cooling core (c), and sandwich clamp assemblies (d), casting alignment card (e).

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.

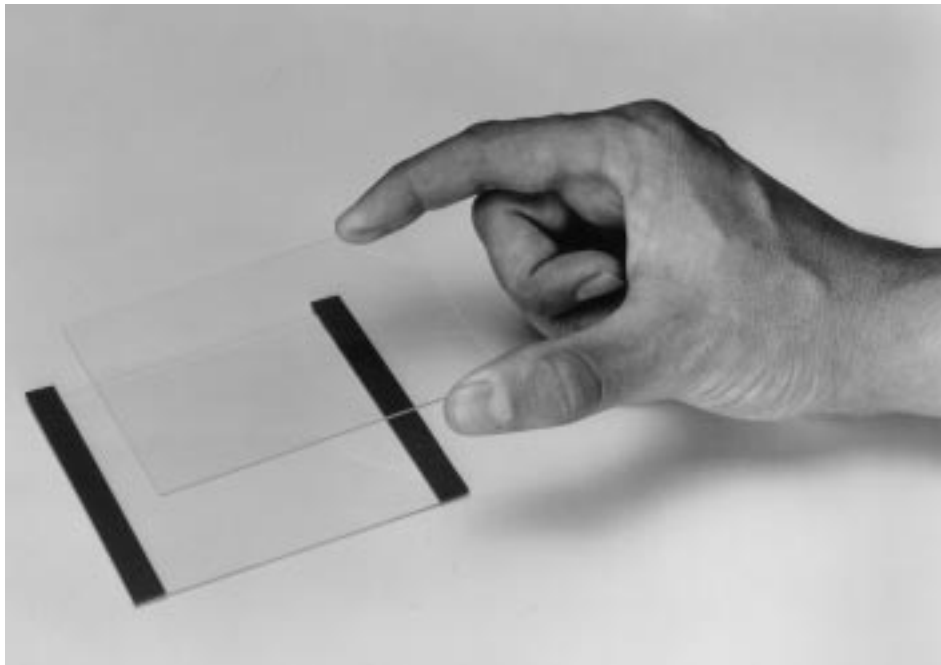


Fig 3.1

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.

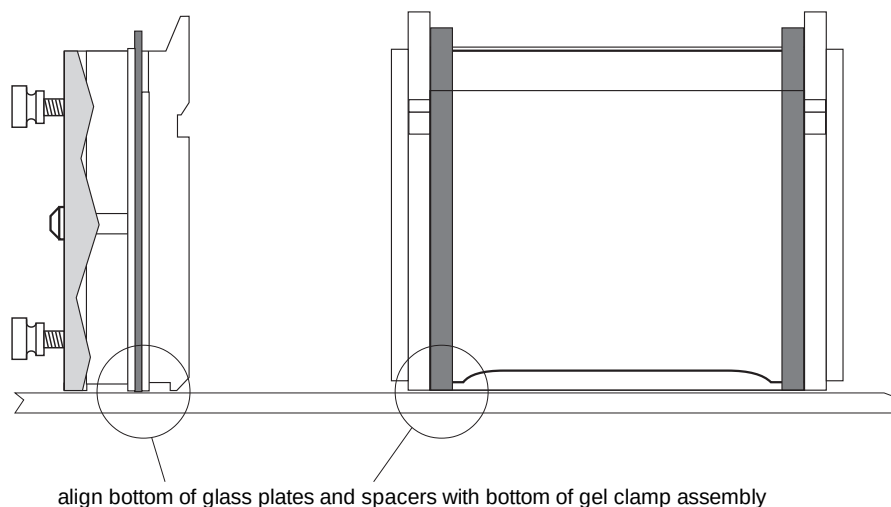


Fig. 3.2

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.

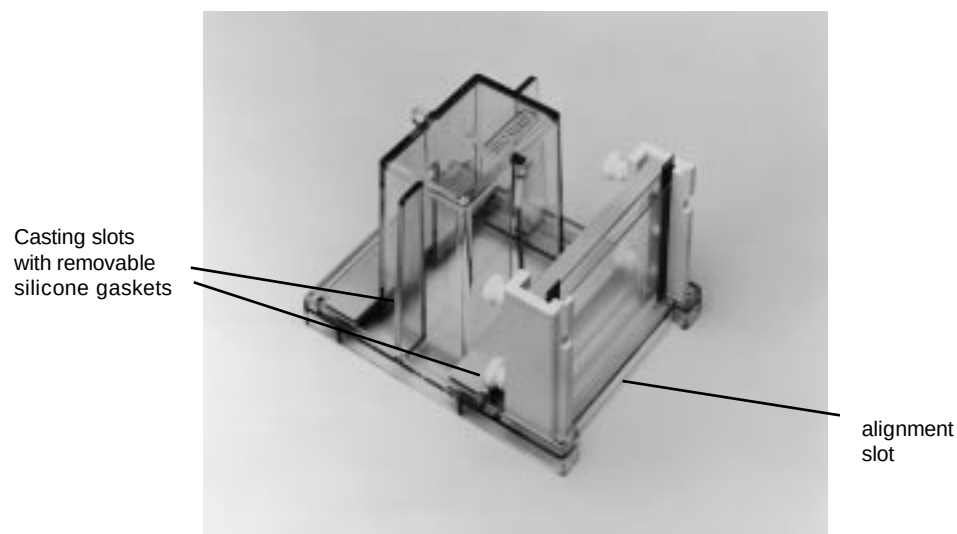


Fig. 3.3

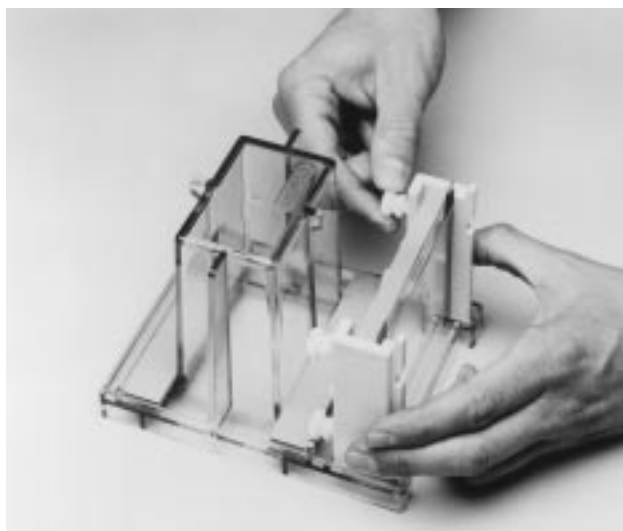


Fig. 3.4

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

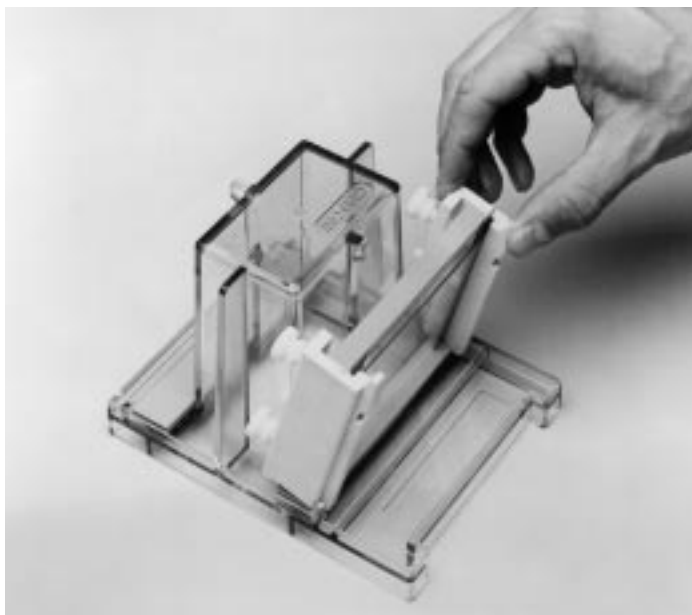
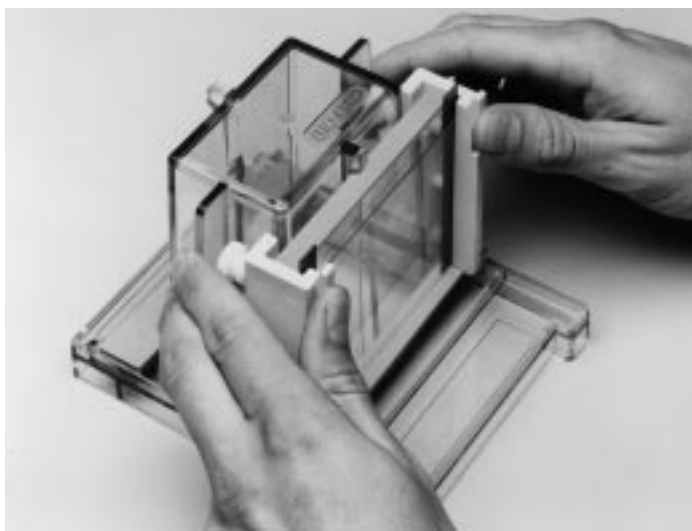


Fig 3.6



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 ($\sim 10^\circ$) 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.)

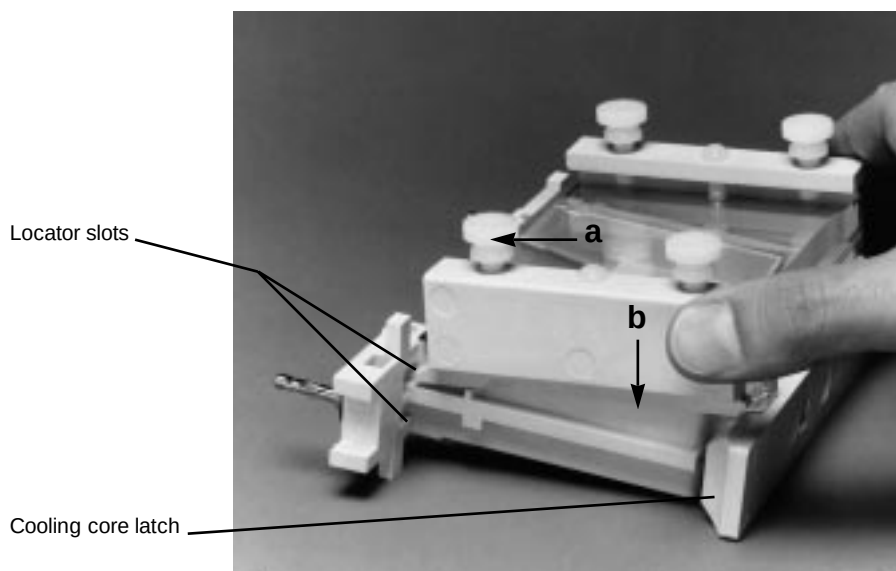


Fig 5.1

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.

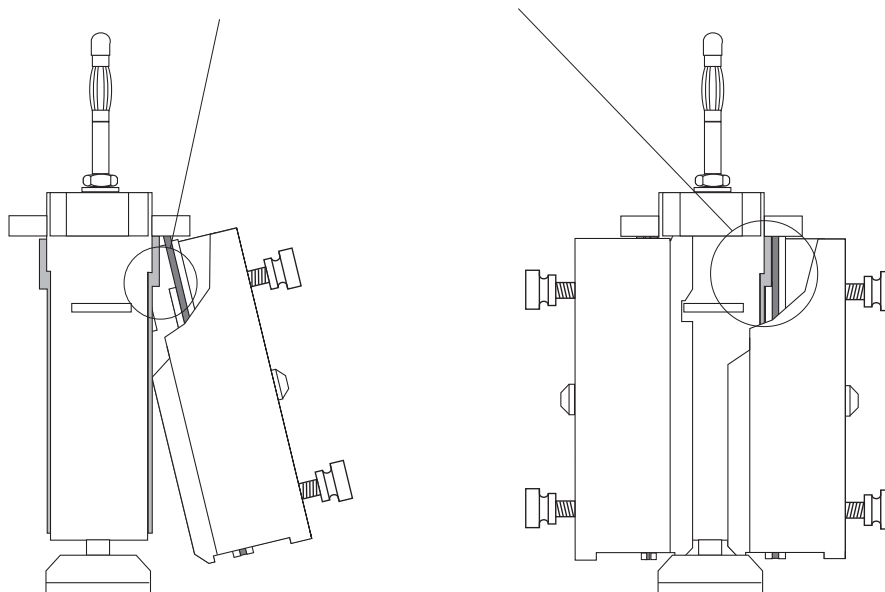


Fig 5.2

(correct alignment of plate and gasket)

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 ($V=I \cdot R$), 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. b. Power conditions excessive.	a. Decrease power setting, or fill lower chamber to within 1 cm of top of short glass plate.
2. Diffuse tracking dye.	a. Decomposition of sample solution and/or buffer stock solutions. b. BPB concentration too high.	a. Prepare fresh reagents. Maximum shelf life of aqueous solutions is 30 days at 4° C for buffer and monomer stocks. b. Remake sample buffer w/BPB concentration at 0.025%.
3. Vertical streaking of protein.	a. Sample overload. b. Sample precipitation.	a. Dilute sample, selectively remove predominant protein in the sample, or reduce voltage by about 25% to minimize streaking. 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 b. Diffusion during migration through the stacking gel. c. Ionic strength of sample lower than that of gel.	a. Minimize the time between sample application and power start up. b. Increase % T of stacking gel to 4.5% or 5% T, or increase current by 25% during stacking.* c. Match ionic strength of sample buffer to that of the gel.
5. Skewed or distorted bands.	a. Poor polymerization around sample wells. b. Salts in sample. c. Uneven gel interface.	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. Remove salts by dialysis, desalting column, etc. 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. b. Low current. c. Excessive salt in sample.	a. Check buffer protocol, dilute if necessary. b. Increase voltage by 25-50%.
8. Run too fast, poor resolution.	a. Running or reservoir buffer too dilute. b. Voltage too high.	a. Check buffer protocol, concentrate if necessary. 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.*
11. Upper buffer chamber leaks.	b. Protein degradation. a. Upper buffer chamber over filled. b. Improper assembly .	a. Keep level of buffer below the top of the long glass plates. b. Be sure core gasket is clean, free of cuts, and lubricated with buffer. Be sure short glass plate is <i>under</i> the notch on the gasket, not on top of it.
12. Leaking during gel casting.	a. Improper assembly of gel sandwich. b. Chipped glass plates.	a. Use the alignment card and check to be sure the spacers and glass plate bottoms are flush prior to pouring gel. 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).

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

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

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 ddH₂O.

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	<u>0.4 ml</u>
	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-HCl < pH 8.8	2.5 ml	2.5 ml
10% (w/v) SDS stock	100 μ l	100 μ l
(store at room temperature)		
Acrylamide/Bis (30% stock) (Degas for $\geq$ 15 minutes at room temperature)	4.0 ml	2.5 ml
*10% ammonium persulfate (fresh daily)	50 μ l	50 μ l (0.05%)
TEMED	5 μ l	5 μ l (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-HCl, pH 6.8	2.5 ml	
10% (w/v) SDS	100 μ l	
Acrylamide/bis (30% stock)	1.33 ml	
10% ammonium persulfate (fresh daily)	50 μ l	(.05%)
TEMED	10 μ l	(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	Silver Stain
Procedure	Stain 1/2 hour with 0.1% Coomassie blue R-250 in fixative (40% MeOH, 10% HOAc) 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	μ g 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 Gasket , 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 (<i>continued</i>)	
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

13.1 General References

1. Bollag, D., Edelstein, S., "Protein Methods", Wiley-Liss, Inc. New York (1991).
2. Allen, R. C., Saravis, C. A., Maurer, H. R., "Gel Electrophoresis and Isoelectric Focusing of Proteins," Walter de Gruyter, Berlin (1984).
3. Andrews, A. T., "Electrophoresis Theory Techniques and Biomedical and Clinical Applications", 2nd edition, Clarendon Press, Oxford (1990).
4. Hames, B. D., Rickwood, D., "Gel Electrophoresis of Proteins - A Practical Approach", 2nd edition, IRL Press, Oxford (1990).
5. Hames, B. D., Rickwood, D., "Gel Electrophoresis of Nucleic Acids - A Practical Approach", 2nd edition, IRL Press, Oxford (1990).

13.2 Native Gel Systems References

1. Ornstein, L. and Davis, B. J., Ann. N. Y. Acad. Sci., 121, 321 (1964).
2. Reisfeld, R. A., Lewis, U. J. and William, D. E., Nature, 195, 281 (1962).
3. Bio-Rad Labs, "A Guide to Native PAGE," Bulletin 1822.

13.3 SDS Gel Systems References

1. Laemmli, U. K., Nature, 227, 680 (1970).
2. Weber, K. and Osborn, M., J. Biol. Chem., 224, 4406 (1969).
3. Neville, D. M., J. Biol. Chem., 246, 6328 (1971).

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