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PROTEAN[®] II xi Cell and PROTEAN II xi 2-D Cell Instruction Manual

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Note

To insure best performance from the PROTEAN II xi 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 PROTEAN II xi 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 _____

Warranty Period _____

Serial No. _____

Warranty

Bio-Rad Laboratories warrants the PROTEAN II xi 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 after confirming the model and serial number of your instrument.

Section 1 General Information

1.1 Introduction

The PROTEAN II xi cell is a vertical slab electrophoresis instrument which combines versatility with practicality. When used with the various combs, spacers, and accessories available, the PROTEAN II xi cell is suitable for most common electrophoretic techniques, including SDS electrophoresis, two-dimensional (2-D) electrophoresis, native electrophoresis, and agarose gel electrophoresis. The PROTEAN II xi cell can run up to 4 slab gels or 16 tube gels simultaneously. The basic unit accommodates gels 16 or 20 cm long. The 20 cm gels offer increased resolution capability, which is especially useful in 2-D applications.

The central cooling core of the PROTEAN II xi cell assures even heat distribution over the entire gel length, permitting excellent resolution with minimal band distortion. Only 1.5 liters of buffer are required. The raised electrode position insures safe operation even with extended overnight runs.

The unique single-screw sandwich clamps allow rapid assembly of the gel sandwiches, while providing even pressure distribution along the entire gel length. This even pressure minimizes the risk of breaking the glass plates, a common problem with multi-screw clamps. The PROTEAN II xi alignment card helps keep spacers upright during sandwich alignment. The combination of the clamps, alignment card, and the casting stand permits assembly and casting of gels in minutes, with little effort. After casting, the completed gel sandwich attaches to the central cooling core with a single motion.

The PROTEAN II xi cell is the instrument of choice for 2-D electrophoresis. With the optional tube adaptors, one can run the first dimension IEF tube gel, and then overlay this gel onto the second dimension SDS slab gel. Thus, the complete 2-D procedure can be done with one dedicated instrument.

1.2 Specifications

Construction:

Cooling core and tube gel adaptor	molded polysulfone
Lid and lower buffer chamber	molded polycarbonate
Clamps, casting stand, and cams	glass and Teflon [®] -filled molded polycarbonate

Electrical leads

flexible, coiled

Electrodes

platinum, 0.010 inch diameter (0.254 mm)

Shipping weight

11 kg (24 lb, 3 oz)

Overall size

26 cm (L) x 19 cm (W) x 30 cm (H)

Gel size

16 x 16 cm slab or 16 x 20 cm slab
1 to 6 mm diameter tube gels

Glass plate sizes

16 cm cell: 16 x 20 cm (inner plate)
18.3 x 20 cm (outer plate)

Cooling core, maximum flow rate

2 liter/min

Maximum coolant temperature

Do not exceed 50 °C

Voltage limit

1,000 volts DC

Teflon is a registered trademark of E. I. DuPont de Nemours and Co.

Note: PROTEAN II xi cell components are not compatible with ethanolamine, ethylene diamine, chlorinated hydrocarbons (e.g., chloroform), aromatic hydrocarbons (e.g., toluene, benzene), or acetone. Use of such organic solvents voids all warranties. Cyanoacrylate and other adhesives will also attack the cell components. Contact your local Bio-Rad office for compatibility information before using any adhesive or organic solvent with this cell.

1.3 Safety



Power to the PROTEAN II xi cell and PROTEAN II xi 2-D 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 these cells are:

1000 VDC 	maximum voltage limit
80 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.**

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 modifications of the instrument not performed by Bio-Rad or an authorized agent.

*IEC 1010-1 is an internationally accepted electrical safety standard for laboratory instruments.

Section 2 Description of Major Parts

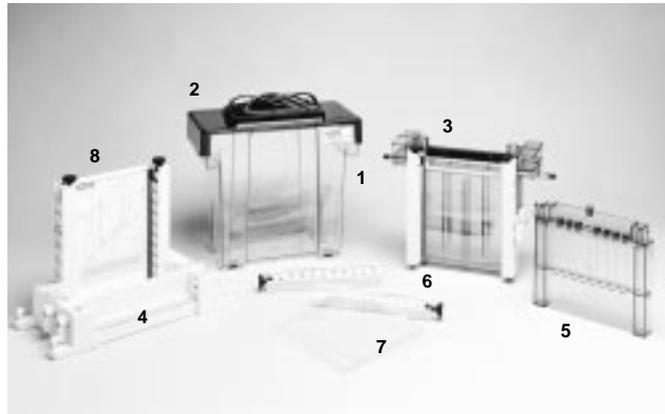


Fig. 2.1. PROTEAN II xi 2-D cell including tube adaptor. 1. Lower buffer chamber, 2. Lid with electrical leads in place, 3. Cooling core with glass plate sandwich attached and core caps in place, 4. Casting stand with glass plate sandwich in alignment slot, 5. Tube gel adaptor, 6. Sandwich clamps, 7. Buffer dam, 8. Alignment card.

2.1 Central Cooling Core

The central cooling core provides the cooling capability which prevents thermal band distortion during electrophoretic separations. The cooling core can be connected to any circulating cooling source. The serpentine flow pattern assures even heat distribution over the entire gel area. An ethylene glycol:water (20:80) solution is recommended as coolant. Other coolants may damage the plastic during extended exposure. If a circulating bath is not available, the core can be connected to a tap water line, or simply filled with 1.8 liters of coolant and plugged to act as a heat sink during electrophoresis.

The central cooling core has a raised upper electrode which is housed in a protective casing, and the lower electrode is recessed to prevent accidental damage.

2.2 Sandwich Clamps

The unique PROTEAN II xi sandwich clamps consist of a single screw mechanism which makes assembly, alignment, and disassembly of the gel sandwich an effortless task. The clamps exert an even pressure over the entire length of the glass plates, providing a leak-proof seal and preventing plate damage due to uneven pressure. Each pair of clamps consists of a left clamp and a right clamp. The sandwich clamps can accommodate up to two 1.5 mm thick gels.

2.3 Casting Stand

The casting stand is separate from the PROTEAN II xi cell so that two gel sandwiches can be cast while others are being run. The one-piece molded unit has two casting slots and one alignment slot. Gel sandwiches are assembled, aligned, and cammed into the stand quickly, without the use of grease.

Double gels may also be cast. Two 1.5 mm (or thinner) gels may be cast in each sandwich, so that up to four 1.5 mm gels can be run at once.

2.4 Upper Buffer Chamber

The completed gel sandwich attaches to the central cooling core so that the outer plate of the sandwich forms the side of the upper buffer chamber. The inner plate is clamped against a rubber gasket on the central cooling core to provide a greaseless, leak-free seal for the upper buffer. Each sandwich forms one side of the cathode chamber. Tube gel adaptors also snap onto the central cooling core to form the upper buffer chamber walls (one adaptor per side). If only one gel is to be run, an upper buffer dam is attached to the core to form the complete upper buffer chamber. The upper buffer chamber will hold approximately 350 ml of buffer when full.

2.5 Lower Buffer Chamber

The lower buffer chamber of the PROTEAN II xi cell encloses the unit and provides stability during electrophoresis. The molded unit requires a minimum buffer volume of only 1.1 liters for 20 cm plates, while providing excellent heat exchange through the central cooling core.

2.6 Lid

Combined with the lower buffer chamber, the lid acts to fully enclose the PROTEAN II xi cell during electrophoresis, thus providing electrical insulation. The lid cannot be removed without disconnecting the electrical circuit. It can be placed on the lower chamber in only one alignment, so that the anode and cathode connections cannot be accidentally reversed. The lid also holds the coiled electrical leads when not in use.

2.7 Tube Gel Adaptor

The tube gel adaptor clamps onto the central cooling core in one easy motion and provides a leak-proof seal for the upper buffer chamber at voltages up to 1,000 V (especially useful for 2-D applications). The molded construction produces a lightweight, yet durable adaptor unit, which has a gel tube locator at the bottom to hold the tubes in a vertical orientation. Each adaptor can hold up to 8 tubes (from 1.0 mm ID to 6 mm ID); 16 tube gels can be run at once using two tube adaptors.

Note: The upper buffer dam may not be used opposite a tube gel adaptor.

2.8 Alignment Card

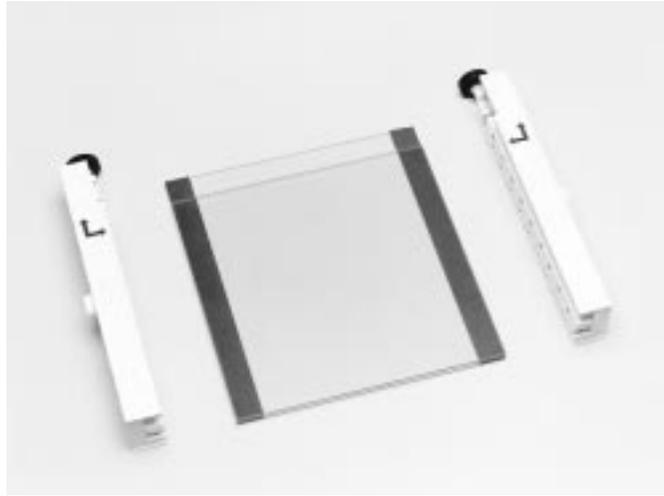
The alignment card greatly simplifies sandwich assembly by keeping the spacers upright during sandwich alignment. A sandwich is assembled by placing two spacers on top of the large outer plate. The alignment card is placed between the two spacers, and the shorter inner plate is then placed on top. Following attachment of the clamps, the sandwich assembly is transferred to the alignment slot of the casting stand for final adjustments.

Section 3 Assembling the Glass Plate Sandwiches

3.1 Assembling Single Sandwiches

Note: Instructions for assembling 16 cm and 20 cm sandwiches are identical, except, of course, for the lengths of the components. To insure proper alignment, make sure all plates and spacers are clean and dry before assembly. The PROTEAN II xi plate washer/holder simplifies glass plate washing and also makes an ideal storage system for clean, dry glass plates. Each plate holder will accommodate up to 8 PROTEAN II xi plates or up to 18 Mini-PROTEAN® II plates.

1. Assemble the gel sandwich on a clean surface. Lay the long rectangular plate down first, then place two spacers of equal thickness along the long edges of the rectangular plate. Next, place a short plate on top of the spacers so that it is flush with one end of the long plate.
2. Locate both the right and left sandwich clamps, and loosen the single screw of each by turning counterclockwise. Place each clamp by the appropriate side of the glass plate stack, with the locating arrows facing up and toward the glass plates.



3. Grasp the whole glass plate sandwich firmly with your right hand. With your left hand guide the left clamp onto the sandwich so that the long and short plates fit the appropriate notches in the clamp. Tighten the single screw enough to hold plates in place.



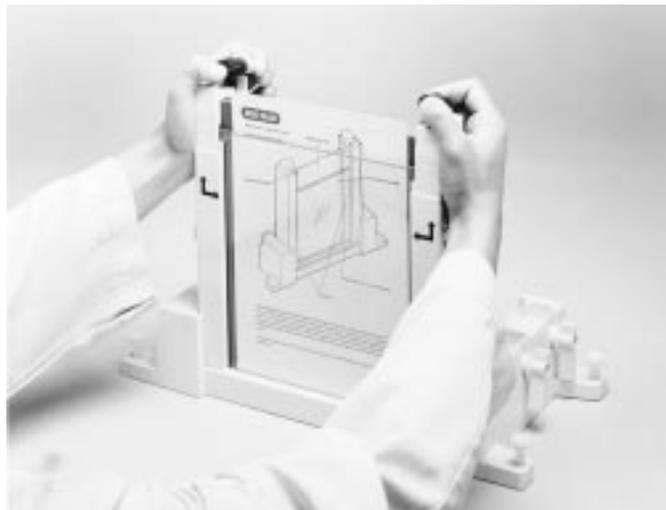
4. Place the right clamp on the right side of the plates, and tighten the clamp screw.
5. Level the casting stand on a flat surface with the alignment slot facing you. Check to see that gaskets are clean and free of residual acrylamide to insure a good seal. Place a flat, grey, silicone gasket in each casting slot.

Note: Always use the alignment slot to properly orient the gel sandwich. Failure to use this slot for alignment can result in casting leaks while pouring the gel or buffer leaks during the run.

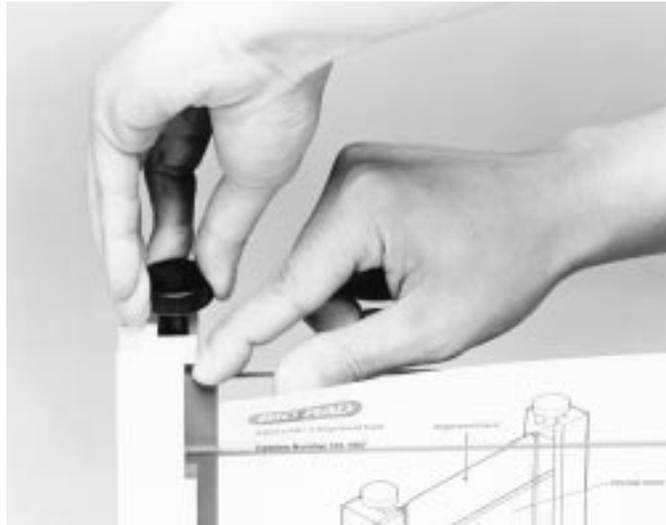
6. Place the assembled gel sandwich in the alignment slot of the casting stand. Loosen the clamp screws, and allow the plates and spacers to align at the surface of the alignment slot. Insert a PROTEAN II xi alignment card between the two glass plates to keep the spacers upright while additional alignment adjustments are made. As an alternative, the alignment card can be positioned between the glass plates when the sandwich is first assembled as in step 1.



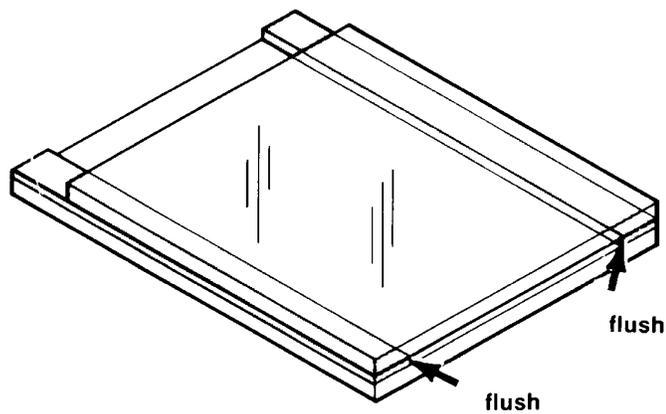
7. Simultaneously push inward on both clamps at the locating arrows, and tighten both clamp screws just enough to hold the sandwich in place. Pushing inward on both clamps at a point below the locating arrows will insure that the spacers and glass plates are flush against the sides of the clamps.



- Loosen one clamp screw slightly. While pushing down on the spacer with one finger, tighten the clamp screw finger-tight with the other hand (see photo). This will insure proper sealing when solution is poured. Tighten the other clamp screw in the same manner. It is important to visually inspect the sandwich while it is in the alignment slot to insure that there are no gaps between the glass plates and the surface of the alignment slot.



- Remove the alignment card. Pull the gel sandwich from the alignment slot. **Check that the plates and spacers are flush at the bottom.** If not, realign the gel sandwich as in steps 6-8.



Note: The easiest way to check for proper alignment is to run a fingernail across the contact area between the glass plates and spacer. If your fingernail catches or drops as you move from plate to spacer to plate, you must realign the sandwich before proceeding to step 10.

10. The cams on the casting stand should be handle up and pulled out. Place the aligned sandwich into one of the casting slots with the longer plate facing you (and the arrows on the clamp facing away from you). When the sandwich is placed correctly, push the cams in, and turn them 180° so that the handles of the cams point downward. The sandwiches are now ready for gel casting.



3.2 Assembling Multiple or “Double-up” Gel Sandwiches

Up to four gels can be run at one time by doubling up gel sandwiches (i.e., 2 gels/side). Double gels are assembled, aligned, and cammed in a manner very similar to that described for single gels.

Note: In order to run four gels instead of two, it is necessary to order two notched inner glass plates and a set of four spacers of equal length.

1. Lay down a long rectangular plate, two spacers, and a notched inner plate.

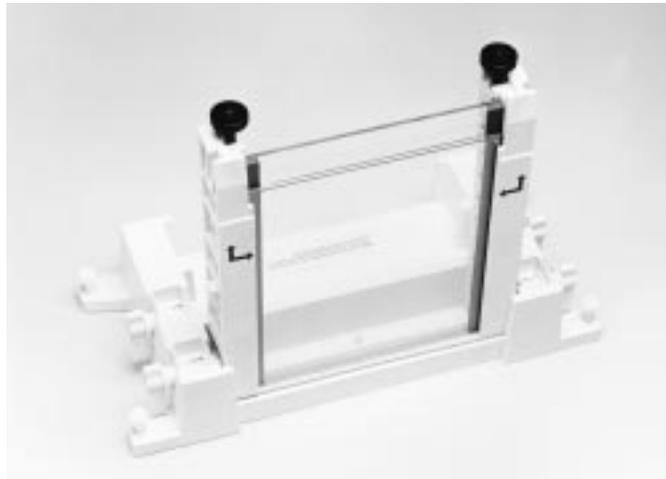


2. Place two more spacers on top of the notched inner plate.

Place the short inner glass plate on top of this set of spacers to form the complete double sandwich.



3. Apply the sandwich clamps as described in Section 3.1, steps 3-4. Insert two alignment cards between each of the sandwiches to keep the spacers upright during sandwich alignment. Align, and then cam the whole assembly into the casting stand. The sandwiches are now ready for gel casting.



Section 4 Casting the Gels

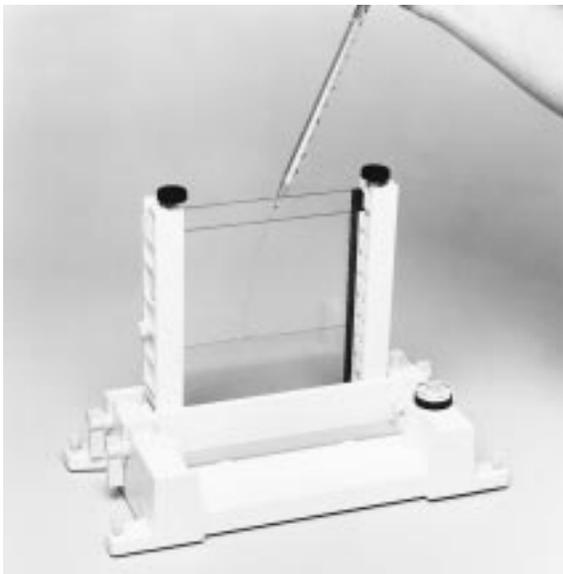
4.1 Casting Discontinuous (Laemmli) Gels

Discontinuous gels consist of a resolving or separating (lower) gel and a stacking (upper) gel. The stacking gel acts to concentrate large sample volumes, resulting in better band resolution than is possible using the same volumes on a gel without a stack. Molecules are then completely separated in the resolving gel. The most popular discontinuous buffer system is that of Laemmli.* This formulation is included in the Appendix.

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

1. Prepare the monomer solution by combining all reagents except ammonium persulfate (APS) and TEMED (see Section 14.1 for formulations). Deaerate the solution under vacuum for at least 15 minutes.

2. Place a comb completely into the assembled gel sandwich. With a marker pen, place a mark on the glass plate 1-2 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 deaerated monomer solution, and pour the solution to the mark, using a glass pipet and bulb. The easiest way to pour is to flow the solution down the middle of the outside plate of the gel sandwich. Another way to pour is to flow the solution down the side of one of the spacers. An alternative method is to use a syringe and Tygon tubing to load the solution from near the bottom of the sandwich. In all cases, 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 using a needle and syringe, using a steady, even rate of delivery to prevent mixing.
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 dehydration of the top of the gel will occur.

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 diluted stock solution B (see Section 14.1) 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 deaerate under vacuum at least 15 minutes.
7. Dry the area above the separating gel completely with filter paper before pouring the stacking gel.

- 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 pouring the monomer solutions.



- 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.



Generally, an overlay solution is not necessary for polymerization when a comb is in place.

- Allow the gel to polymerize 30-45 minutes. Remove the comb by pulling it straight up slowly and gently.
- Rinse the wells completely with distilled water. The gels are now ready to be attached to the central 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.

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. No overlay solution is necessary.
4. Let the gel polymerize for 45 minutes to 1 hour. The gel is now ready to load and run. Remove the comb, and rinse thoroughly with distilled water.

4.3 Casting Gradient Gels

Polyacrylamide concentration gradients, made with an external gradient former (Model 385, catalog number 165-2000), can be introduced into the PROTEAN II xi gel sandwich assembly either from the bottom or from the top. A peristaltic pump is required for introduction from the bottom. Introduction from the top can be done by gravity flow or with a peristaltic pump. Follow the gradient former instructions for formulating the gradient. If gradients are pumped into the gel sandwich from the bottom, the low density solution (low percent monomer) must enter first. If the gradient enters from the top, the high density solution (high percent monomer) enters first.

Pouring Gradient Gels from the Bottom

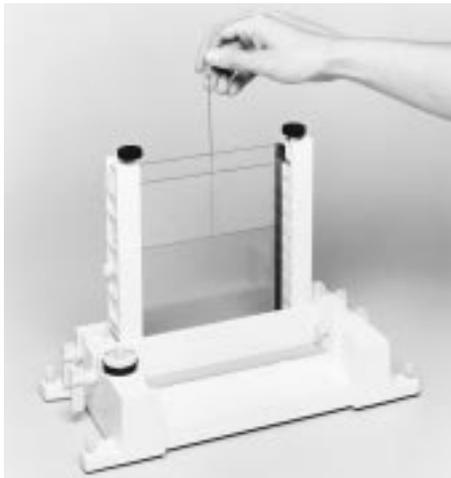
1. After assembling the gel sandwich as described in Section 4, attach the gradient former tubing to a gradient pouring needle (catalog number 165-2007).
2. Turn the gel sandwich to the casting stand, turn the casting stand on its side, locate the bottom filling ports directly under the space in the sandwich, and insert the bottom-fill needle through the rubber gasket, so that it protrudes about 2 mm into the sandwich. Make sure the needle opening faces one of the glass plates.



3. Stand the casting stand up on a level surface, add APS and TEMED to the monomer solutions, and begin pumping the gradient monomer solution.
4. Once the gradient is poured (this should take no more than 10 minutes from the time the initiators are added to the first monomer solution), withdraw the needle from the gasket, and immediately clean the gradient former, tubing, and needle by pumping distilled water through them.
5. Overlay the monomer solution (see Section 4.1), or insert the comb (see Section 4.2), depending on whether the gel is discontinuous or continuous, respectively.

Pouring Gradient Gels by Top-Filling

1. Attach the gradient maker tubing to a needle (catalog number 165-1943), a long cannula, or a piece of tubing that will fit between the glass plates of the gel sandwich.
2. Insert the needle, cannula, or tubing as far as possible into the sandwich, and center it.
3. Add APS and TEMED to the monomer solution, and begin pumping the gradient. As the monomer level in the sandwich approaches the tip of the needle, withdraw the needle slowly, so that it always stays above the monomer level.



4. Immediately rinse any remaining monomer out of the needle by pumping water through the gradient former and out the needle.
5. Overlay the gel, or insert the comb, as outlined in Sections 4.1 and 4.2, respectively.

4.4 Casting Agarose Gels

Agarose gels in vertical apparatus may slide down between the glass plates. To prevent this, one of the two plates used to form the agarose gel sandwich should be a frosted inner plate (catalog number 165-1825 for 16 cm gels and catalog number 165-1826 for 20 cm gels).

1. Assemble the glass plate sandwich as outlined in Section 3, using one frosted plate and one regular clear plate. The frosted plate should be the shorter inner plate (the plate on the inside during running). This will allow visualization of the tracking dye during the run. Make sure that the frosted side of the plate is on the inside of the gel sandwich.
2. Place the sandwich assembly in a warm air incubator (50 °C) for 5-10 minutes before casting the gel. This will prevent premature gelling of the agarose.
3. Cam the warm assembly to the casting stand.

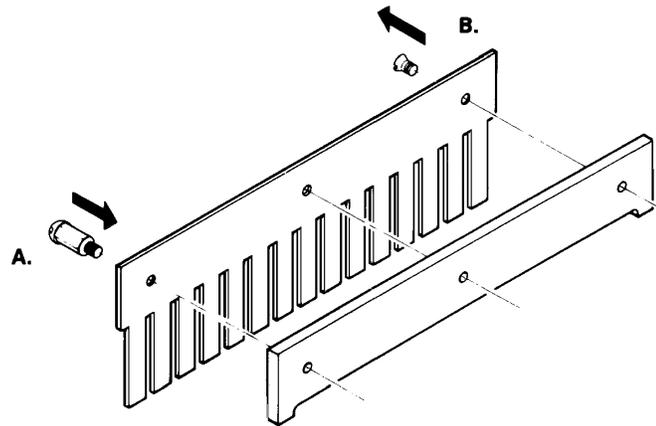
4. Immediately pour the molten (55 to 60 °C) agarose. One convenient method of pouring is to tilt the sandwich assembly and pour agarose directly down the long rectangular glass plate.



Note: If an incubator is not available, molten (75 to 85 °C) agarose can be poured into a room temperature sandwich assembly. This temperature is high enough to prevent premature gelling of the agarose, yet low enough to prevent cracking of the glass plates.

5. Insert the prewarmed comb carefully.
6. Allow the agarose to cool to ambient temperature before use.
7. Remove the comb very slowly to avoid tearing the gel.

Note: There are some special tricks to properly remove a comb from a vertical agarose gel. The most important point is to introduce water or buffer under the comb while it is being removed. To introduce buffer or water, use a squirt bottle or a needle and syringe to force fluid under the teeth of the comb while it is slowly removed from the gel. Another option is to insert the comb only partway into the gel. This can easily be done with the aid of comb conversion screws (catalog number 165-1859). The three standard screws on the comb are replaced with the three large head comb conversion screws, with the protruding head of the conversion screws resting on top of the longer outer glass plate. The well depth of the comb is limited to 10 mm instead of the standard 25 mm well depth.



A. Comb conversion screw. B. Standard comb screw.

Section 5 Assembling the Upper Buffer Chamber

5.1 Assembly

1. Lay the central cooling core down flat on a lab bench. Make sure the core caps are in place on the cooling core ports.
2. Seat the white U-shaped gasket onto the core with the flat (non-stepped) side down.

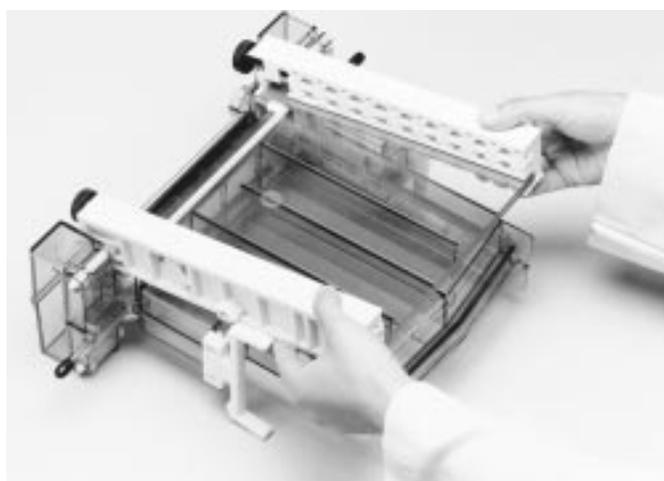
Note: To help insure a good upper buffer seal with your gaskets for the PROTEAN II xi cell, lubricate the entire front of the gaskets (the shaded portion) with water or upper buffer prior to attaching the gel sandwich to the central cooling core. This will allow the glass plate sandwich to slide onto the gasket properly.



3. After the gel is cast and the comb is removed (if applicable), release the gel sandwich from the casting stand by turning the cams to the “up” position and pulling them outward. Pull the gel sandwich straight out of the stand.



4. With the short glass plate facing the cooling core (and the locating decal on the clamps facing the core), position the gel sandwich so that the grooves in the upper portion of the clamps are fitted onto the locating pins on the central cooling core.* The gel sandwich should be positioned at an angle of $\leq 20^\circ$ with the cooling core. Keeping this angle to a minimum and lubricating the gasket will prevent distortion of the gasket while the sandwich slides into place.



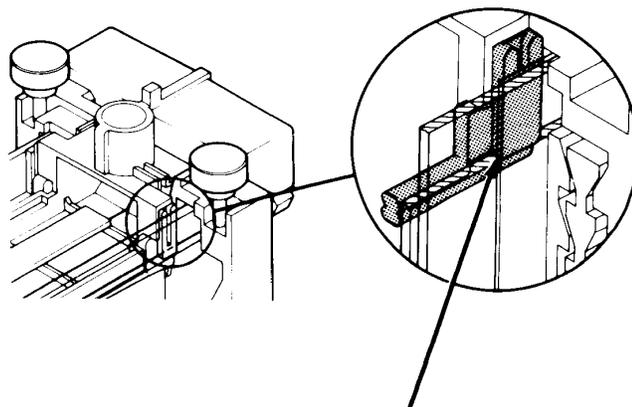
***Note:** The locating pins on the central cooling core must be tightly secured in place to insure optimal pressure during operation. If these pins are vibrated loose during transport or repeated use, the decrease in pressure on the clamp can be enough to allow the cell to leak. If the pins are loose, they can be gently tightened using a coin or screwdriver.

5. With your fingers below the latch on the cooling core and your thumbs resting on the clamps, gently push the gel sandwich onto the cooling core with one simple motion. The upper edge of the short inner glass plate should be butted against the notches of the U-shaped gasket, and the tabs of each clamp should be held securely against the latch assemblies on both sides of the cooling core.



6. Turn the central cooling core to its other side, and repeat steps 1 through 5 to attach the second gel sandwich to it.

Note: When the gel sandwich has been properly installed, the shorter inside glass plate will be forced against the notch in the U-shaped gasket to create a leak-proof seal. Always inspect the contact between the gasket and glass plate to make sure the glass plate is butted against the notch in the gasket and is not resting on top of or below this notch. Improper installation of the gel sandwich can result in buffer leakage during the run. As a standard procedure, pour buffer into the upper buffer compartment, and check for buffer leaks prior to a run. In addition, we recommend marking the level of the upper buffer on the glass plates prior to electrophoresis. Checking this level after 1-2 hours will show whether a slow leak is occurring. This is especially important when the electrophoresis cell is being used for overnight experiments.



5.2 Use of the Buffer Dam

If only one gel is to be run, the acrylic buffer dam must be attached to the cooling core on the other side to form the complete upper buffer chamber. Position the acrylic plate between two clamps by matching the notches on the clamps to the notches on the acrylic plate (as in Section 3.1, steps 2-3), and then slide the dam up each clamp as far as possible. No further alignment is necessary. The acrylic buffer dam fits both 16 and 20 cm clamps.

Note: We do not recommend the use of the acrylic buffer dam at elevated temperatures as the block may warp and cause buffer leaks.



Note: Failure to slide the dam up completely to the top of the clamp will result in an upper buffer leak.

Section 6 Loading the Samples

Sample loading can be done in one of three ways. The most common method is to load liquid samples into wells formed by casting a gel with a well-forming comb. The second method uses the entire gel surface as a single well for liquid samples. The third method involves placing a tube gel or gel strip over the entire gel surface, as in 2-D procedures.

6.1 Loading of Sample Wells

The approximate sample volumes that each well will hold for all available combs is included in Section 13.2.

1. Prepare 1.5 liters of electrode buffer. Set aside 350 ml for the upper buffer chamber.
2. Pour 325-350 ml of electrode buffer into the upper buffer chamber. At this point, check the integrity of the upper buffer seal. If the buffer appears to be leaking, remove the gel sandwich assemblies, re-lubricate the gasket, and then re-attach the gel sandwich assemblies (as in Section 5).
3. Place the remainder of the electrode buffer into the lower buffer chamber. Lower the central cooling core into the lower buffer chamber at a slight angle to prevent air entrapment under the gel sandwich. A few, isolated bubbles under the gel will not affect the run. With 16 cm plates, it is necessary to dilute the lower buffer with distilled water to a level of 1 cm above the bottom of the gel plates. Be sure to mix the lower buffer well with a stir bar on a magnetic stirrer.

Note: Dilution of the lower buffer by up to 1:2 with dH₂O will have no detrimental effect on resolution. Dilution of the upper buffer is not recommended.

4. Load the samples into the wells under the electrode buffer with a Hamilton syringe. 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 glycerol in order to underlay the sample in the well without mixing.

5. An easier method of sample loading is to use an Eppendorf-like pipettor and disposable tips. To accomplish this successfully, use the optional beveled short plate (catalog number 165-1827 for 16 cm and 165-1828 for 20 cm) so that you can insert the pipet tip further into the well before sample delivery. This will prevent inter-well mixing of samples. Or, use the Bio-Rad Prot-Elec tips (catalog number 223-9915 or 223-9917), which are designed for loading samples into wells.

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. After electrophoresis, the sample is transferred electrophoretically to a membrane, which then can be cut into several identical strips for analysis. Follow the instructions for casting the separating portion of a discontinuous gel (Section 4.1), except pour the gel nearly to the top of the sandwich. Allow just enough room for sample loading. (A stacking gel may also be added to this type of gel.)

1. Prepare electrode buffer, and add to lower reservoir as in Section 6.1. Place the central cooling core in the lower chamber, and add electrode buffer to the upper reservoir chamber.
2. The sample may be loaded with an Eppendorf-type pipettor, with a needle and syringe, or with a 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 closely as possible (1-2 mm) to the surface of the gel.

6.3 Gels as Samples

All two-dimensional techniques involve this variation of sample loading. Either a cylindrical gel or a strip of a slab gel may be placed on top of a slab gel for separation into a second dimension. This procedure is described in detail in Section 10.

Section 7 Running the Gel

1. Place the lid on top of the lower buffer chamber to enclose the PROTEAN II xi cell fully. Note that the lid can be placed only in one orientation, so that the anode and cathode connections cannot be reversed.
2. Attach the electrical leads to a suitable power supply, such as those in Section 13.6, with the proper polarity (this connection could accidentally be reversed).
3. Apply power to the PROTEAN II xi cell and begin electrophoresis. As a safety precaution, always set voltage, current, and power limits when possible. See the Appendix (Section 14.4) for specific running conditions.

Section 8 Set-up Options

8.1 Buffer Recirculation

Buffer recirculation is sometimes necessary for extended electrophoretic runs or for use with certain weak buffer systems.

To recirculate electrode buffer from the lower to the upper chambers, a few simple modifications have to be made to the PROTEAN II xi cell.

1. Locate the recirculation port tabs on the lid of the cell. The tab directly above the upper buffer chamber and one of the tabs directly above the lower buffer chamber must be removed. Remove by grasping with a pair of pliers and twisting the tab until it breaks off (see photo).



2. Locate the punch out tab in the upper right corner on both sides of the central cooling core.

Carefully bore one of the thin membrane tabs with a drill and 1/4 in (6 mm) bit.

3. Insert a section of tubing down through the port above the lower buffer chamber and to the bottom of the lower buffer chamber.
4. Run this tubing through a peristaltic pump and into the upper buffer chamber through its port.
5. Buffer is then pumped from the lower to the upper buffer chamber. As the upper chamber fills, buffer will overflow through the port on the core back into the lower buffer chamber.

The buffer flowing through the tubing and pump is electrically active. For this reason, handle the tubing carefully when the power supply is on. Do not touch any exposed liquid with the power supply on. Tube connections should be made with the power supply turned off. Both the recirculation pump and the recommended power supplies must be ground isolated by design to minimize the potential for shock hazard. However, working around high voltage equipment in a laboratory environment is potentially dangerous. As a result, it is the user's responsibility to always exercise care in setting up and running electrophoresis instruments. **If a liquid leak occurs, always turn off the power supply before correcting the problem.**





Note: Recirculation can only be used with continuous buffer systems (i.e., systems in which the anode buffer and cathode buffer are the same).

8.2 Cooling Options

The cooling core may be used in any of the following ways:

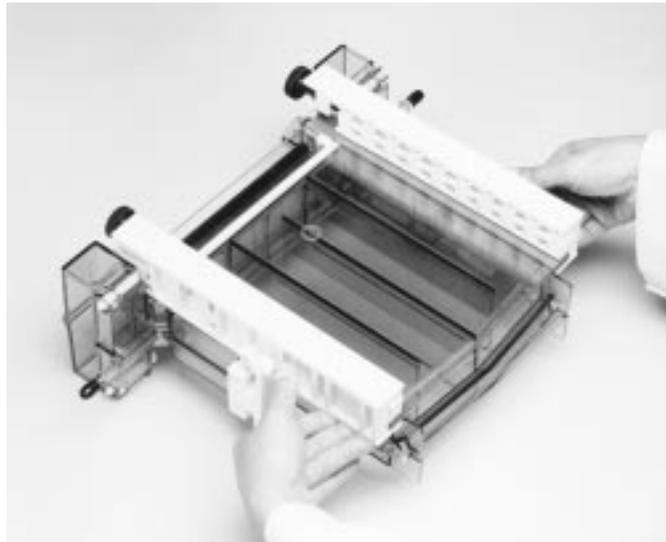
1. The core can be connected to any circulating cooler. Any common anti-freeze or ethylene:glycol (20:80) may be circulated through the core. Do not use ethanol or methanol for coolant. For most SDS-PAGE and 2-D applications temperature should be set between 10 and 15 °C. For some specialized applications employing native or non-denaturing gel systems, in which temperature sensitive enzymes or other labile proteins are to be separated, the system can be cooled to 2-4 °C. For these applications, greatest heat transfer efficiency is achieved by circulating coolant through the core at 2 °C and filling the lower buffer chamber to the top of the slab gels. The lower buffer is continually circulated by stirring.
2. The core may be filled with coolant and the circulation ports plugged off with the core caps. The coolant will act as a heat sink during electrophoresis. This option works especially well for SDS-PAGE.
3. Distilled tap water may be circulated through the core.
4. Temperature of the coolant can be monitored by placing a thermometer through one of the ports in the lid of the PROTEAN II xi cell. The temperature of the upper and lower buffers will equilibrate to the temperature of the coolant in about 1/2 hour. Remove the tab in the lid as in Section 8.1, step 1.
5. During periods of non-use, the cooling core can be left filled with coolant or water and capped.

Section 9

Removing the Gels

1. After electrophoresis is complete, turn off the power supply, and disconnect the electrical leads.
2. Disconnect coolant hoses from the core (if applicable), and plug off the ports.
3. Remove the cell lid, and carefully pull the central cooling core out of the lower chamber. Pour off the upper buffer.
4. Lay the central cooling core on its side, and remove the sandwich assembly in the following manner:

With your index fingers below the sandwich clamps and your thumbs resting on the latches in the cooling core, gently remove the assembly by pulling up toward you (in a manner opposite to the way it was attached in Section 5). Pull the sandwich assembly off the locating pins on the top of the cooling core.



5. Loosen the single screw of each clamp, and remove the clamps from the glass plates.
6. Push one of the spacers of the sandwich out to the side of the plates without removing it.
7. Gently twist the spacer so that the upper glass plate pulls away from the gel.



8. Remove the gel by gently grasping two corners of the gel and lifting off, or alternatively, place the gel and glass plate under fixative solution, and agitate gently until the gel separates from the glass plate.
9. If the gel is to be stained later, place it in a suitable container with fixative solution (e.g., 40% methanol/10% acetic acid). See Section 14.5 for staining formulations. If the proteins on the gel are to be electrophoretically transferred to a membrane, place the gel in equilibration buffer (do not put in fixative).

Note: The Model 556 Gel Destainer (catalog number 165-2010) is ideal for rapid destaining (less than 1 hour) of Coomassie blue stained gels.

Section 10

Two-Dimensional Electrophoresis

Two-dimensional electrophoresis can provide exceptionally high resolution of the protein components in a complex sample. It is capable of resolving several thousand individual protein species. Based on the method of O'Farrell, the first dimension is isoelectric focusing (IEF), during which proteins are separated according to their isoelectric points. The second dimension is SDS-polyacrylamide gel electrophoresis, in which proteins are separated on the basis of their molecular size. Since O'Farrell's original work, many variations of the 2-D procedure have been reported which may also be used. The following procedure is based on the work of Dr. Denis Hochstrasser.⁶⁻⁷ The flow chart (Section 10.1) outlines in sequence the essential steps of 2-D electrophoresis and refers to the solution protocols in Section 14.

Note: This section focuses on 2-D electrophoresis. The PROTEAN II xi cell may also be used for one-dimensional tube gel electrophoresis. One can adapt this protocol to any of the common electrophoretic techniques, using either continuous or discontinuous buffer systems, by following the instructions for casting, loading, and running tube gels.

10.1 Sequence of Steps for 2-D Protocol

Step	Time Interval
Day 1	
1. Pour tube gels	polymerize 2 hours
2. Prepare electrolytes, prepare and load samples	½ hour-1 hour
3. Electrophorese at 200 V constant voltage	2 hours
4. Electrophorese at 500 V constant voltage	2 hours
5. Electrophorese at 800 V constant voltage	16 hours (overnight)
6. Cast slab gels for second dimension gels while first dimension gels are running	1 hour
7. Prepare second dimension running buffer	10 minutes
Day 2	
8. Disassemble tube apparatus	2 minutes
9. Extrude gels from tubes and overlay tube gels onto slab gels	25 minutes
10. Electrophorese the second-dimension SDS gel	4-4½ hours

10.2 Protocol for IEF First Dimension

Casting IEF Tube Gels

For reproducible 2-D gels, it is essential that the IEF tube gels be precisely the same length and that polymerization be identical from day to day. Care must be taken in pouring the gels to the same height so that the polymerization height will be the same from tube to tube. An overlay step is not necessary in IEF first dimension tube gels. The meniscus formed on top of the gel will not influence the pH gradient or the resolution of the bands. The advantage of not overlaying is the formation of gels of more uniform length and composition. Stock solutions and formulations for first dimension tube gels are given in Section 14.

1. Mark the capillary tubes (1.5 mm ID, 6.0 mm OD, 180 mm, catalog number 165-3138) with a laboratory marker 14.0 cm from one end.
2. Connect each capillary tube to a 1 ml syringe using a small piece of Tygon tubing 3/16" ID x 1/4" OD, and approximately 2 cm in length (not included). Fill either a test tube rack or a level casting stand, such as Bio-Rad's Model 225 Tube Gel Casting Stand (catalog number 165-2020) with a disposable 12 x 75 mm test tube for each capillary tube. Insert a capillary tube/syringe assembly into each test tube.
3. Prepare the first dimension monomer solution and degas well. (The removal of molecular oxygen by degassing is essential for reproducible polymerization.)

Warning: Always wear gloves to prevent exposure to acrylamide.

4. Add the APS and TEMED, and swirl 8 to 10 times. Working quickly, pipet 1 ml of acrylamide solution into each test tube. Using the syringe, pull up the liquid in each tube to the 14.0 cm mark. Let the capillary tubes sit undisturbed, with syringes attached, for 2 hours at room temperature to allow complete polymerization to occur.

5. After polymerization is completed, remove the capillary tubes from each test tube. Remove the syringe and Tygon tubing. Press and rotate the bottom of the capillary tube squarely down on a piece of Parafilm™ to remove excess acrylamide. Wipe off the excess acrylamide.
6. Inspect the gels before loading; bubbles within the gel prevent focusing and these gels should be discarded.

Note: Alternative methods for filling capillary tubes can be used, such as wrapping the bottom end of the capillary tube with two layers of Parafilm™ laboratory film and filling using a syringe and fine gauge cannula (gel tube loading needle, 165-1943). The cannula should be long enough to reach the bottom of the tube. Slowly inject the acrylamide solution into the bottom of the tube, withdrawing the cannula as the acrylamide enters the tube. Fill to the mark on the tube.

Sample Preparation and Loading

Sample preparation prior to isoelectric focusing is one of the most important steps for obtaining reproducible two-dimensional electrophoresis gels. There is no method which is optimal for every sample, and it may be necessary to experiment with different protein solubilization methods to determine which is best.

1. Prepare the first dimension running solutions as described in Section 14.
2. Prepare the IEF sample concentrate solution A and/or iso-urea solution E as described in Section 14. These solutions should be prepared fresh, or frozen in aliquots.

Note: Sample loads above 400 µg total protein may cause loss of resolution in the second dimension slab gel.

3. Replace the notched white gaskets with the tube adaptor gaskets specifically designed for use during the first dimension of 2-D electrophoresis. Do not lubricate or wet the red tube adaptor gaskets.

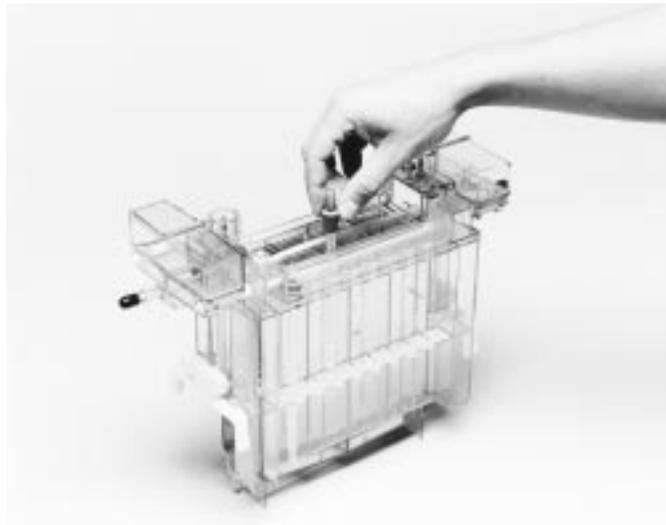
Note: Tube gels have a much higher resistance than slab gels due to their small diameter. Since current seeks the path of least resistance, a current leak may occur if there is an alternative path of conductance such as a wet gasket. A current leak is a safety hazard to the researcher as well as the equipment.

4. Attach the tube gel adaptor to the cooling core in the same manner that a slab gel sandwich is attached (see Section 5.1). Sandwich clamps are not needed to attach the tube adaptor to the core. Finish assembling the upper buffer chamber with a second tube gel adaptor. Because of the higher voltages required for focusing, we recommend always using two tube gel adaptors and not the buffer dam for focusing.



Note: Buffer leakage during isoelectric focusing can result in damage to the cooling core. It is important to check for buffer leaks by monitoring both the current and the upper buffer level. During the course of a normal IEF run, the current decreases as the resistance of the gel increases, the pH gradient is established, and the upper buffer level is maintained. If a buffer leak should occur, the current will increase, and the level of upper buffer may decrease. Do not exceed 1,000 V as the maximum focusing voltage.

5. Insert the tube gels into the tube gel adaptor, using the gel tube locator at the bottom of the unit to align the tubes. Plug any unused tube holes with a stopper.



6. Prepare the sample just before loading. The amount of denaturing sample solution A and/or iso-urea solution E will depend upon the protein concentration of sample and upon the type of sample. An initial starting ratio of 1 μ l IEF sample concentrate for every 10 μ l sample can be used. For denaturing, samples are heated at 95 °C for 5 minutes then cooled for 2 minutes at room temperature before loading or before adding iso-urea solution.
7. Load the samples with a Hamilton syringe, or with a Drummond pipet tip. (Generally, 15 to 30 μ l of final diluted sample is loaded.)

- Carefully overlay the sample with upper electrolyte (20 mM NaOH).

Running the IEF Gels

- Fill the central cooling core with water or coolant. Cap the inlet and outlet port with the caps provided.
- Fill the lower buffer chamber with ~4.5 liters of lower running electrolyte (see Section 14). Place on a level surface or leveling table.
- Lower the cooling core/tube apparatus into the lower chamber of the PROTEAN II xi cell. Lower carefully so as not to introduce any air bubbles under the gel tubes. All bubbles must be removed from the bottom of the tubes to insure proper electrical contact.
- Pour 325-350 ml freshly degassed upper running electrolyte (see Section 14) into the upper buffer chamber, put the lid on, and attach the power cables to the power supply.
- Run the first dimension gels at room temperature with a constant voltage of 200 volts for 2 hours, followed by 500 volts for 2 hours, and then 800 volts overnight (16 hours). As a safety precaution, always set voltage, current, and power limits when possible.

Note: Phycocyanin is a colored protein found in Bio-Rad's IEF standards (catalog number 161-0310). Although these standards cannot be used for pI calibration in 2-D procedures, because denaturation in urea produces too many peptide spots, the phycocyanin is excellent for monitoring the first dimension IEF. It retains its blue color and will focus in a tight blue band when focusing is finished. Loading one tube with the focusing standards is an easy way to monitor the progress of the focusing run.

- Cast the second dimension SDS slab gel during the running of the first dimension (see Section 4).

Note: This protocol does not use a stacking gel. However, if a stacking gel is required for a particular application, it should be cast on a level surface. It is important that the same amount of monomer be used for each stacking gel to insure stacking gels of identical depth. If a comb is not used, as in most 2-D applications, then the stacking gel should be overlaid with 1.0 ml of water saturated sec-butanol. After polymerization is complete, drain off the overlay (or remove the combs), and rinse the gel surface briefly with distilled deionized water.

Extruding Tube Gels

- After electrophoresis, remove the tube gels from the tube gel adaptor. Rinse both the top and bottom of each gel thoroughly with distilled water. Failure to rinse before extrusion will result in residual base (NaOH) or acid (phosphoric) on the gel that will interfere with measurement of the pH gradient. Place the tubes, in order, into the tube rack, and fill each tube to the top with distilled water.
- Attach a long (at least 2 inch), fine (at least 26 gauge), beveled needle (such as catalog number 165-1944) to a 3 ml plastic syringe. Rim the upper and lower few mms of the gel by inserting the needle between the gel and the glass tube (point against the glass wall to prevent tearing of the gel) while forcing distilled water through the syringe and needle. Turn the gel tubes so that the entire circumference is rimmed (see photo). Often tube gels may be extruded without rimming, using water pressure as below.



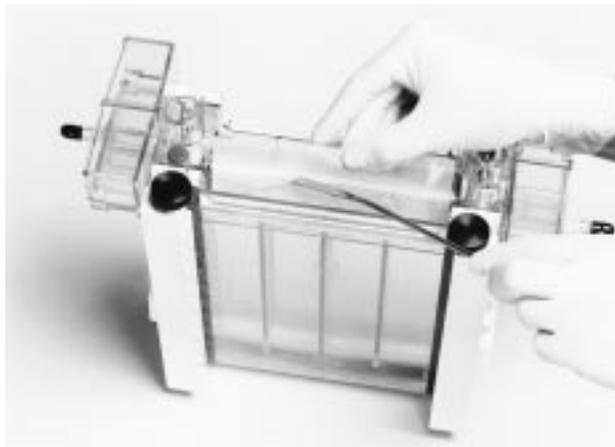
3. Attach a piece of silicone tubing to a 1 ml syringe and to the outside wall of the glass tubes on the top end of the tube. Using the syringe filled with distilled water, apply a firm, even pressure to start the gel extruding from the tubes. As the gel moves further out of the tube, apply less pressure so that rate of extrusion remains constant. Care must also be taken not to extrude too quickly. Only slight pressure is required to remove the last 1-2 cm of gel from the tube.
4. It is convenient to extrude the gels onto longitudinally folded pieces of Parafilm or plastic sheets.
5. Pipet 150 ml of transfer solution along the length of the extruded gel.



Embedding of First Dimension onto Second Dimension

Note: When handling gels, it is advisable to wear gloves. First dimension gels tend to be sticky to the touch and will tear easily.

1. Use of a beveled short glass plate (catalog number 165-1827 for 16 cm cell and 165-1828 for 20 cm cell) in the second dimension slab sandwich will greatly improve the ease of embedding the tube gel on top of the slab gel. Rinse the top of the completed second dimension SDS slab gel thoroughly with distilled water, and drain off excess water. Attach clamp assemblies to cooling core.
2. Using a spatula, direct the gel from the Parafilm to the bevel at the top of the inner glass plate, starting at one side and proceeding across the gel. Place a few drops of SDS electrode buffer along the top of the tube gel.



3. Use a spatula to seat the tube gel on the slab gel. Check that the tube gel is in contact with the slab gel over its entire length. Be sure to remove all air bubbles that are between the tube gel and the slab gel. By placing the tube gel directly on top of the second dimension slab gel, between the glass plates, no agarose overlay is necessary. If the tube gel diameter is greater than the second dimension slab gel thickness, an agarose overlay may be necessary to insure good contact and to prevent the tube gel from slipping off the slab gel. The agarose overlay is 1% agarose in 1x stacking gel buffer, diluted 1:4 (see Section 14.1, solution C).

Note: If you would like to apply molecular weight standards to the second dimension, we recommend using a 2-D comb for casting the stacking gel. As an alternative, if a stacking gel is not desired, you can make a tube gel with a mixture of agarose and Bio-Rad standards. Then simply cut the agarose into pieces, and load a piece directly on top of the second dimension slab gel in tandem with the IEF tube gel.

4. Electrophorese the SDS slab gel as in Section 7.
5. Remove the gels as in Section 9.
6. Stain the gels as in Section 14.5.

Note: Tube gels may be frozen for future use or applied directly to a second dimension slab gel. To freeze a tube gel, place the gel lengthwise in a stoppered tube in an EtOH:dry ice bath. Once frozen, gels can be stored at -20 °C.

Section 11

Maintenance of Equipment

PROTEAN II xi cell chamber, 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 (if 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. A less toxic alternative is 5% KOH in 100% methanol.
Glass tubes	After use, rinse with laboratory detergent solution, scrub out if possible, then rinse with distilled H ₂ O. Store glass tubes in chromic/sulfuric acid solution until next use. Then rinse thoroughly with distilled water and dry in forced air or vacuum oven before use.

Warning: Exercise extreme caution for acid cleaning wear safety glasses, a lab coat, and rubber gloves. Keep a container of NaCO₃ nearby to neutralize spills.

Section 12

Troubleshooting Guide – PAGE, SDS-PAGE, 2-D IEF/SDS-PAGE

Problem	Cause	Solution
1. “Smile” effect — band pattern curves upward at both sides of the gel.	<ul style="list-style-type: none"> a. Center of the gel running hotter than either side. b. Power conditions excessive. 	<ul style="list-style-type: none"> a. Fill inner core with coolant. b. Circulate coolant at 10-15 °C. c. Decrease power setting.
2. Diffuse tracking dye.	<ul style="list-style-type: none"> a. Decomposition of sample solution and/or buffer stock solutions. b. Diffusion. 	<ul style="list-style-type: none"> a. Prepare fresh reagents — maximum shelf life of aqueous solutions is 30 days at 4 °C for buffer and monomer stocks. b. If protein bands are diffuse as well as to the tracking dye, increase current by 25-50% and/or increase % T of resolving gel.*
3. Vertical streaking of protein.	<ul style="list-style-type: none"> a. Sample overload. b. Sample precipitation. 	<ul style="list-style-type: none"> a. Dilute sample, selectively remove predominant protein in the sample, or reduce current by about 25% to minimize streaking. b. Centrifuge sample or decrease % T of resolving gel.*
4. Horizontal streaking (2-D gels).	<ul style="list-style-type: none"> a. Incomplete solubilization prior to first dimension. b. Interfering nucleic acids in sample. 	<ul style="list-style-type: none"> a. If urea/nonionic detergent is not sufficient, use SDS as in Ref. 15.5-2. Centrifugation of sample may be necessary (up to 100,000 x g for 30 minutes) to remove undissolved particulates. b. Treat sample with DNase or RNase as in Ref. 15.5-1.
5. Broad or diffuse protein bands or spots (2-D).	<ul style="list-style-type: none"> a. Diffusion due to slow migration. b. Chemical changes due to ionic contaminants in urea. 	<ul style="list-style-type: none"> a. Increase current by 20%. b. Deionize urea.
6. Lateral band spreading.	<ul style="list-style-type: none"> a. Diffusion out of the wells prior to turning on the current. b. Diffusion during migration through the stacking gel. 	<ul style="list-style-type: none"> 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.*

Troubleshooting Guide (continued)

Problem	Cause	Solution
7. Skewed or distorted bands.	<ul style="list-style-type: none"> a. Poor polymerization around sample wells. b. Salts in sample. c. Uneven gel interface. 	<ul style="list-style-type: none"> a. Degas stacking gel solution thoroughly prior to casting; increase ammonium persulfate and TEMED concentrations by 25%, or add riboflavin phosphate to 5 µg/ml in addition to the usual catalyst levels in the stacking gel. b. Remove salts by dialysis, desalting column, etc. c. Increase reaction rate, overlay carefully.
8. Run taking unusually long time.	<ul style="list-style-type: none"> a. Buffers too concentrated. b. Low current. 	<ul style="list-style-type: none"> a. Check buffer protocol, dilute if necessary. b. Increase current by 25-50%.
9. Run too fast, poor resolution.	<ul style="list-style-type: none"> a. Buffer too dilute. b. Current too high. 	<ul style="list-style-type: none"> a. Check buffer protocol, dilute if necessary. b. Decrease current by 25-50%.
10. Doublets observed where a single protein species is expected (SDS-PAGE).	<ul style="list-style-type: none"> a. A portion of the protein may have been re-oxidized during the run or may not have been fully reduced prior to run. 	<ul style="list-style-type: none"> a. Prepare fresh sample buffer solutions if over 30 days old; increase β-mercaptoethanol concentration in the sample buffer.
11. Observe fewer bands than expected and one heavy band at dye front.	<ul style="list-style-type: none"> a. More than one band migrating at the dye front. 	<ul style="list-style-type: none"> a. Increase % T of resolving gel.*
12. Nonlinear pH gradient (at basic end).	<ul style="list-style-type: none"> a. Upper electrolyte depleted 	<ul style="list-style-type: none"> a. Increase the concentration of upper electrolyte to 100 mM

*Polyacrylamide gels are described by reference to two characteristics:

- 1) The **total** monomer concentration (%T)
- 2) The **crosslinking** monomer concentration (%C)

$$\%T = \frac{\text{gm Acrylamide} + \text{gm Bis-Acrylamide}}{\text{Total Volume}} \times 100$$

$$\%C = \frac{\text{gm Bis-Acrylamide}}{\text{gm Acrylamide} + \text{gm Bis-Acrylamide}} \times 100$$

Section 13 Equipment and Accessories

13.1 PROTEAN II xi Cell Configurations

Product Description	Catalog Number
<i>PROTEAN II xi Cells</i>	
PROTEAN II xi 16 cm Cell*	165-1801
1.5 mm spacer (4), 15 well comb (2)	165-1802
1.0 mm spacer (4), 15 well comb (2)	165-1803
0.75 mm spacer (4), 15 well comb (2)	165-1804
PROTEAN II xi 20 cm Cell*	165-1811
1.5 mm spacer (4), 15 well comb (2)	165-1812
1.0 mm spacer (4), 15 well comb (2)	165-1813
0.75 mm spacer (4), 15 well comb (2)	165-1814

All PROTEAN II xi cells include the central cooling core with gaskets and core caps, lower buffer chamber, lid with power cables, 2 sets of glass plates, 4 sandwich clamps, 4 spacers, 2 combs, an upper buffer dam, a casting stand with gaskets, a leveling bubble, alignment card, and instructions.

*Cells contain all of the above except spacers and combs (order separately).

13.2 Accessories

Product Description	Catalog Number	
	16 cm cell	20 cm cell
<i>Glass Plates*</i>		
Inner Plate (2)	165-1821	165-1823
Outer Plate (2)	165-1822	165-1824
† Frosted Inner Plate (2) (agarose gels)	165-1825	165-1826
† Beveled Inner Plate (2) (2-D procedures)	165-1827	165-1828
** Notched Inner Plate (1) (double-up procedures)	165-1832	165-1833

***Note:** One complete gel sandwich consists of 1 outer (long) plate, 1 inner (short) plate, and 2 spacers.

** One complete “double-up” sandwich (2 gels/side) consists of 1 outer plate, 1 inner plate, 1 notched inner plate, and 4 spacers. See Section 3.2 for assembly.

†Used in conjunction with regular outer plate.

PROTEAN II Plate Washer/Holder

Plate Washer System , includes: Plate Holders (2), Tank and Lid	165-1991
Plate Holder (1)	165-1992

Product Description	Catalog Number	
	16 cm cell	20 cm cell
<i>Spacers (set of 4)</i>		
0.5 mm	165-1841	165-1846
0.75 mm	165-1842	165-1847
1.0 mm	165-1843	165-1848
1.5 mm	165-1844	165-1849
3.0 mm	165-1845	165-1850
<i>Sandwich Clamps</i>		
16 cm Set (one left, one right)	165-1901	
20 cm Set (one left, one right)	165-1902	
<i>Optional and Replacement Parts</i>		
Central Cooling Core , with gaskets	165-1806	
Lower Buffer Chamber	165-1807	
Cell Lid , with safety cables	165-1808	
Upper Buffer Dam (1)	165-1909	
Slab Casting Stand , with gaskets	165-1911	
Replacement Gaskets , for casting stand (2)	165-1912	
Gradient Pouring Needle (for bottom filling) (2)	165-2007	
Replacement Gaskets , central cooling core (2)	165-1913	
Alignment Card	165-2029	

Product Description	Catalog Number
<i>Combs (each)</i>	
25 well x 0.75 mm , 60 µl volume	165-1861
25 well x 1.0 mm , 80 µl volume	165-1862
25 well x 1.5 mm , 120 µl volume	165-1863
20 well x 0.5 mm , 54 µl volume	165-1865
20 well x 0.75 mm , 82 µl volume	165-1866
20 well x 1.0 mm , 110 µl volume	165-1867
20 well x 1.5 mm , 164 µl volume	165-1868
20 well x 3.0 mm , 328 µl volume	165-1869
15 well x 0.5 mm , 74 µl volume	165-1870
15 well x 0.75 mm , 110 µl volume	165-1871
15 well x 1.0 mm , 147 µl volume	165-1872
15 well x 1.5 mm , 221 µl volume	165-1873
15 well x 3.0 mm , 442 µl volume	165-1874
10 well x 0.5 mm , 114 µl volume	165-1875
10 well x 0.75 mm , 172 µl volume	165-1876
10 well x 1.0 mm , 229 µl volume	165-1877
10 well x 1.5 mm , 343 µl volume	165-1878
10 well x 3.0 mm , 687 µl volume	165-1879
5 well x 1.0 mm , 522 µl volume	165-1882
5 well x 1.5 mm , 783 µl volume	165-1883
5 well x 3.0 mm , 1.57 ml volume	165-1884
3 well x 1.5 mm , 1.37 ml volume	165-1888
Blank x 0.75 mm , 2.44 ml volume	165-1891
Blank x 1.0 mm , 3.26 ml volume	165-1892
Blank x 1.5 mm , 4.88 ml volume	165-1893
Blank x 3.0 mm , 9.76 ml volume	165-1894
2-D x 1.0 mm , 1 ref. well, 28 µl (ref. well) volume	165-1897
2-D x 1.5 mm , 1 ref. well, 42 µl (ref. well) volume	165-1898
2-D x 3.0 mm , 1 ref. well, 84 µl (ref. well) volume	165-1899
Teflon Comb Conversion Screws* (10)	165-1859

* To convert any PROTEAN II xi comb with a standard 25 mm well depth to a 10 cm well depth. May be used for agarose or acrylamide gels.

Glass Tubes

Product Description				
ID mm	OD mm	Length mm	Pkg. Qty.	Catalog Number
1.0	5.0	180	24	165-3136
1.5	6.0	150	24	165-3137
1.5	6.0	180	24	165-3138
2.0	6.5	180	24	165-3139
2.4	4.0	160	24	165-3155
3.0	5.0	125	24	165-3150
5.0	7.0	125	24	165-3122

13.3 PROTEAN II xi 2-D Cells

Product Description	Catalog Number
PROTEAN II xi 2-D Cell , 1.0 mm, 16 cm	165-1931
PROTEAN II xi 2-D Cell , 1.5 mm, 16 cm	165-1932
PROTEAN II xi 2-D Cell , 1.0 mm, 20 cm	165-1933
PROTEAN II xi 2-D Cell , 1.5 mm, 20 cm	165-1934

PROTEAN II xi 2-D cells include central cooling core, lower buffer chamber, lid with power cables, 2 sets of glass plates (with bevel), 4 sandwich clamps, 24 glass tubes (tube diameter = spacer thickness), 2 tube gel adaptors, 16 stoppers, 16 grommets, 2 2-D combs, 4 spacers, 1 upper buffer dam, slab casting stand, leveling bubble, alignment card, and instructions.

13.4 Accessories

Product Description	Catalog Number
Tube Gel Adaptor , with a complete set of grommets (4-8 mm O.D. tubes) and stoppers	165-1940
Tube Gel Adaptor Replacement Gaskets , 2	165-1947
Stoppers , 8	165-1941
Grommets and Stoppers for 4-5 mm OD Tubes , 12	165-1984
Grommets and Stoppers for 6-7 mm OD Tubes , 12	165-1985
Gel Tube Loading Needle , 18 cm, 22 gauge, blunt tip, Luer hub (for casting monomer in small diameter tubes)	165-1943
Gel Tube Extrusion Needle , 9 cm, 26 gauge, beveled tip, Luer hub (for removing gels from tubes)	165-1944
PROTEAN II xi Multi-Gel Casting Chamber	165-2025

13.5 Electrophoresis Chemicals

Product Description	Quantity per Package	Catalog Number
Acrylamide, 99.9%	100 g	161-0100
Acrylamide, 99.9%	500 g	161-0101
Acrylamide, 99.9%	1 kg	161-0107
Acrylamide, 99.9%	2 kg	161-0103
Preweighed Acrylamide/Bis, 37.5:1 mixture	30 g	161-0122
Preweighed Acrylamide/Bis, 37.5:1 mixture	150 g	161-0125
Bis (N,N'-Methylene-bis acrylamide)	5 g	161-0200
Bis (N,N'-Methylene-bis acrylamide)	50 g	161-0201
Piperazine di-Acrylamide (PDA)	10 g	161-0202
Tris	500 g	161-0716
Tris	1 kg	161-0719
Glycine	250 g	161-0717
Glycine	1 kg	161-0718
Boric Acid	500 g	161-0750
Boric Acid	1 kg	161-0751
SDS (Sodium dodecylsulfate)	25 g	161-0300
SDS (Sodium dodecylsulfate)	100 g	161-0301
SDS (Sodium dodecylsulfate)	1 kg	161-0302
Ammonium Persulfate	10 g	161-0700
Dithiothreitol	1 g	161-0610
Dithiothreitol	5 g	161-0611
2-mercaptoethanol	25 ml	161-0710
TEMED	5 ml	161-0800
TEMED	50 ml	161-0801
Agarose, Standard Low – m_r	100 g	162-0100
CHAPS	1 g	161-0460
CHAPSO	1 g	161-0465
Agarose, Standard Low – m_r	500 g	162-0102
SDS-PAGE Standards, 14,400-97,400 MW		161-0304
SDS-PAGE Standards, 45,000-200,000 MW		161-0303
Prestained SDS-PAGE Standards, low range		161-0305
Prestained SDS-PAGE Standards, high range		161-0309
2-D SDS-PAGE Standards		161-0302
Silver Stain SDS-PAGE Standards, low range		161-0314
Silver Stain SDS-PAGE Standards, high range		161-0315
Silver Stain Plus Kit , includes fixative enhancer concentrate, silver complex solution, reduction moderator solution, image development reagent, development accelerator reagent, complete instructions and Material Safety Data Sheets		161-0449

13.5 Electrophoresis Chemicals (continued)

Product Description	Quantity per Package	Catalog Number
Coomassie Blue R-250	10 g	161-0400
Bromophenol Blue	10 g	161-0404
Triton X-100	500 ml	161-0407
Urea	250 g	161-0730
Urea	1 kg	161-0731
Bio-Lyte® Ampholytes		
Bio-Lyte 3/10 Ampholyte, 40%	10 ml	163-1112
Bio-Lyte 3/5 Ampholyte, 20%	10 ml	163-1132
Bio-Lyte 4/6 Ampholyte, 40%	10 ml	163-1142
Bio-Lyte 5/7 Ampholyte, 40%	10 ml	163-1152
Bio-Lyte 6/8 Ampholyte, 40%	10 ml	163-1162
Bio-Lyte 7/9 Ampholyte, 40%	10 ml	163-1172
Bio-Lyte 8/10 Ampholyte, 20%	10 ml	163-1182
Large Volume Ampholytes		
Bio-Lyte 3/10 Ampholyte, 40%	25 ml	163-1113
Bio-Lyte 4/6 Ampholyte, 40%	25 ml	163-1143
Bio-Lyte 5/7 Ampholyte, 40%	25 ml	163-1153
Bio-Lyte 5/8 Ampholyte, 40%	25 ml	163-1193
Bio-Lyte 6/8 Ampholyte, 40%	25 ml	163-1163
AG® 501-X8 Mixed Bed Ion Exchange Resin	100 g	143-7424

13.6 Power Supplies

Product Description	Catalog Number
PowerPac 3000, 100/120 VAC	165-5056
PowerPac 3000, 220/240 VAC	165-5057
Model 1000/500 Power Supply, 100/120 VAC	165-4710
Model 1000/500 Power Supply, 220/240 VAC	165-4711

Section 14

Appendix

14.1 Reagents and Gel Preparation for SDS-PAGE Slab Gels (Laemmli buffer system*)

Stock Solutions

- A. Acrylamide/Bis (30% T, 2.67% C)
146 g acrylamide (29.2 g/100 ml)
4 g N'N'-Bis-methylene-acrylamide (0.8 g/100 ml)
Make to 500 ml with distilled water. Filter and store at 4 °C in the dark (30 days maximum).
Or substitute Bio-Rad's Preweighed Acrylamide/Bis
37.5:1 mixture (Catalog No. 161-0112, 30 g)
(Catalog No. 161-0106, 200 g)
150 g Acrylamide/Bis (30 g/100 ml) to 500 ml with dH₂O
- B. 1.5 M Tris-HCl, pH 8.8
54.45 g Tris base (18.15 g/100 ml)
~150 ml distilled water
Adjust to pH 8.8 with 1 N HCl. Make to 300 ml with distilled water and store at 4° C.
- C. 0.5 M Tris-HCl, pH 6.8
6 g Tris base
~ 60 ml distilled water
Adjust to pH 6.8 with 1 N HCl. Make to 100 ml with distilled water and store at 4° C.
- D. 10% SDS
Dissolve 10 g SDS in water with gentle stirring and bring to 100 ml with dH₂O.
- E. Sample Buffer (SDS reducing buffer) (store at room temperature)
- | | |
|------------------------------|--------|
| Distilled water | 3.8 ml |
| 0.5 M Tris-HCl, pH 6.8 | 1.0 ml |
| Glycerol | .80 ml |
| 10% (w/v) SDS | 1.6 ml |
| 2 β-mercaptoethanol | 0.4 ml |
| 0.05% (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 | 45 g | (15 g/l) |
| Glycine | 216 g | (72 g/l) |
| SDS | 15 g | (5 g/l) |
- to 3 liters with dH₂O

Store at 4 °C. Warm to 37 °C before use if precipitation occurs.

Dilute 300 ml 5X stock with 1,200 ml dH₂O for one electrophoresis run.

Do not adjust the pH with acid or base.

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

14.2 Separating Gel Preparation - 0.375 M Tris, pH 8.8

	12% ^a	7.5% ^b
Distilled water	33.5 ml	48.5 ml
1.5 M Tris-HCl, pH 8.8	25.0 ml	25.0 ml
10% (w/v) SDS stock (store at room temperature)	1.0 ml	1.0 ml
Acrylamide/Bis (30% stock) (Degas for ≥15 minutes at room temperature)	40.0 ml	25.0 ml
*10% ammonium persulfate (fresh daily)	500 µl	500 µl (0.05%)
TEMED	50 µl	50 µl (0.05%)
TOTAL MONOMER+	100 ml	100 ml

* To make the 10% ammonium persulfate solution, dissolve 100 mg APS in 1 ml dH₂O.

+ One can prepare any desired volume of monomer solution by using multiples of the 100 ml recipes.

a. For SDS treated proteins in the approximate molecular weight range between 10 and 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 High MW SDS-PAGE Standards (catalog number 161-0303).

Calculated Volumes (in ml) Required Per Gel Slab*

Spacer Thickness	16 cm Length	20 cm Length
0.5 mm	12.8 ml	16 ml
0.75 mm	19.2 ml	24 ml
1.0 mm	25.6 ml	32 ml
1.5 mm	38.4 ml	48 ml
3.0 mm	76.8 ml	96 ml

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

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

	16 cm	20 cm
Distilled water	6.1 ml	12.2 ml
0.5 M Tris-HCl, pH 6.8	2.5 ml	5 ml
10% (w/w) SDS	100 µl	200 µl
Acrylamide/Bis (30% stock) (Degas for 15 minutes at room temperature)	1.3 ml	2.6 ml
10% ammonium persulfate (fresh daily)	50 µl	100 µl (.05%)
TEMED	<u>10 µl</u>	<u>20 µl (0.1%)</u>
TOTAL STOCK MONOMER	10 ml*	20 ml**

*Enough for two 0.75 mm or 0.50 mm gels.

**Enough for two 1.5 or 1.0 mm gels.

1. To prepare the monomer solutions, combine all reagents, except the APS and TEMED, and deaerate 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 of the gels.

14.4 Running Conditions*

We recommend that gels be run under constant current conditions with an appropriate power supply, such as the PowerPac 3000 or Model 1000/500 power supply (see Section 13.6 for ordering information).

Gel Thickness	Current Conditions mA per gel	
	Stacking Gel	Separating Gel
0.5	8	12
0.75	13	18
1.0	16	24
1.5	25	35
3.0	50	70

Run time is between 4 and 5 hours, depending on length of gel. Under constant current conditions, voltage will gradually increase during the run. As a safety precaution, always set voltage, current, and power limits when possible.

*Recommended power conditions for optimal resolution with minimal distortion.

14.5 Comparison of Coomassie Blue and Silver Staining

	Coomassie Blue	Silver Stain
Procedure:	Sensitivity:	Stain 1/2 hour with 0.1% Coomassie Blue R-250 in fixative (40% MeOH, 10% HOAc). Destain with 40% MeOH/10% HOAc to remove background (usually 1 to 3 hrs).

14.6 2-D Stock Solutions

First Dimension IEF Tube Gels

Detergent Solution

0.3 g CHAPS
100 µl Nonidet P-40
900 µl ddH₂O
Dissolve CHAPS in water, then add Nonidet P-40 (NP-40).

First Dimension Capillary Tube Gel Monomer Solution

11 g urea (9.2 M final concentration)
3 ml acrylamide/bis stock (4.5% total monomer)
Stir to dissolve the urea. This step is done slowly without heating or with very gentle heating only.

0.2 ml Bio-Lyte[®] 5/7 ampholyte
0.8 ml Bio-Lyte 3/10 ampholyte
1 ml detergent solution (CHAPS/NP-40) from above

Add deionized water to 20 ml.

Mix and degas.

Add 20 µl TEMED after degassing to avoid evaporation.

Add 40 µl 10% (w/v) APS, swirl 8-10 times and cast the gels.

This makes 20 ml total volume, enough to cast one set of gels using the casting tube.

Sample Solution A

1.0 g SDS
0.232 g DTT or DTE

Dissolve in ddH₂O to a final volume of 10 ml. Store in aliquots at -70 °C.

Iso-Urea Solution E

0.1 g DTT
0.4 g CHAPS
5.4 g urea
500 µl Bio-Lyte 3/10 ampholyte
6 ml ddH₂O

Electrolytes

First Dimension Upper Running Electrolyte-Cathode (20 mM NaOH)

Dissolve 0.4 g NaOH in 500 ml deionized water and degas thoroughly for 30 minutes.

First Dimension Lower Running Electrolyte-Anode (10 mM H₃PO₄)

Dilute 1.8 ml concentrated H₃PO₄ in 2.6 liters deionized water and degas thoroughly for 30 minutes.

Transfer Solution

40 ml 0.5 M Tris-HCl, pH 8.8
80 ml 10% SDS
8 ml 0.05% bromophenol blue
150 ml ddH₂O

14.7 Running Conditions

IEF is carried out at 200 volts constant voltage for 2 hours, followed by 500 volts constant voltage for 2 hours, and finally 800 volts constant voltage for 16 hours (overnight).

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