Mini-PROTEAN® Tetra Cell

Gel Electrophoresis

General Information

1.1 Introduction

The Mini-PROTEAN® Tetra cell runs both hand-cast gels and Ready Gel® precast gels interchangeably. The Mini-PROTEAN Tetra system includes a casting stand and glass plates with permanently bonded gel spacers that simplify hand-casting and eliminate leaking during casting. The cell can run one or four gels, and the mini tank is compatible with other Bio-Rad electrode modules for tank blotting, 2-D electrophoresis, and electroelution.

1.2 Components

To get the best performance from your Mini-PROTEAN Tetra Cell, familiarize yourself with the components by assembling and disassembling the cell before using it (refer to Figures 1 and 2).

Spacer Plate	The spacer plate is the taller glass plate with permanently bonded gel spacers. Spacer plates are available in 0.75 mm, 1.0 mm, and 1.5 mm thicknesses, which are marked directly on each spacer plate.
Short Plate	The short plate is the shorter, flat glass plate that combines with the spacer plate to form the gel cassette sandwich.
Casting Frame	The casting frame, when placed on the benchtop, evenly aligns and secures the spacer plate and the short plate together to form the gel cassette sandwich prior to casting.
Gel Cassette Assembly	One casting frame, a spacer plate, and a short plate form one gel cassette assembly.
Casting Stand	The casting stand secures the gel cassette assembly during gel casting. It contains pressure levers that seal the gel cassette assembly against the casting gaskets.
Gel Cassette Sandwich	A spacer plate and short plate with polymerized gel forms a gel sandwich.
Buffer Dam	The molded, one-piece buffer dam is used when running only one or three gels.

Electrode Assembly	The electrode assembly holds the gel sandwich. It houses the sealing gasket, the upper and lower electrodes, and the connecting banana plugs. The anode (lower electrode) banana plug is identified with a red marker and the cathode (upper electrode) banana plug with a black marker.
Companion Assembly	The companion assembly allows you to run gels 3 and 4. It holds the gel sandwich and houses the sealing gasket.
Mini Tank and Lid	The mini tank and lid combine to fully enclose the inner chamber during electrophoresis. The lid cannot be removed without disrupting the electrical circuit. The mini tank and lid are also compatible with other Bio-Rad electrode modules for blotting, first-dimension of 2-D electrophoresis, and electroelution.

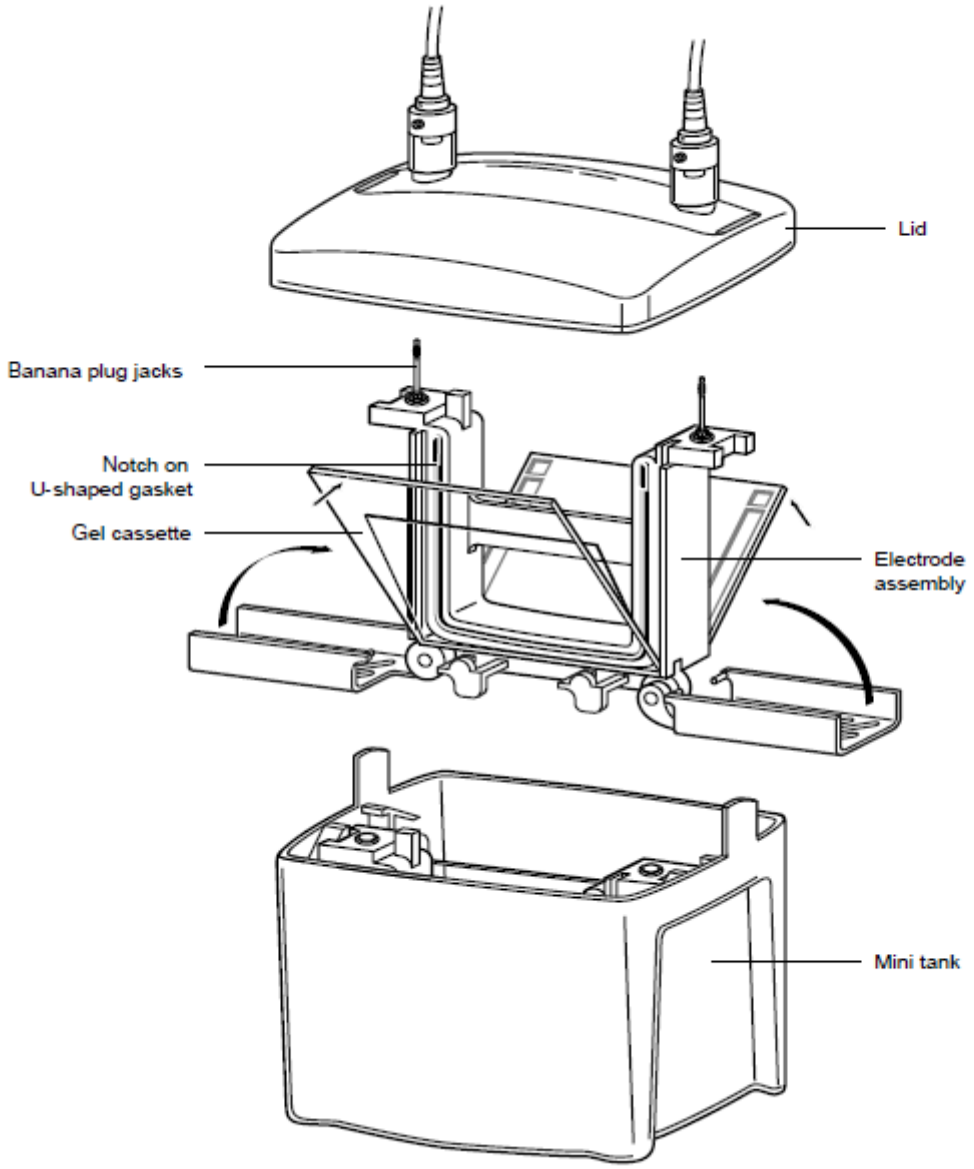

Fig. 1. Assembling the Mini-PROTEAN Tetra Cell.

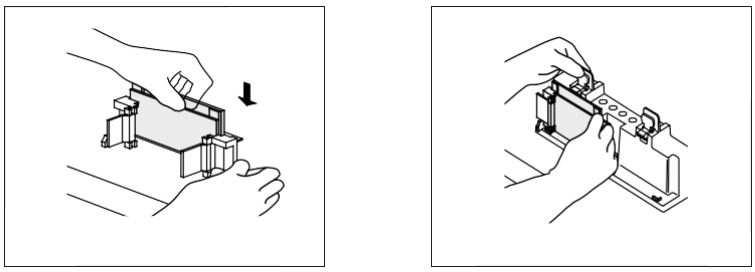

Fig. 2. Assembling the Mini-PROTEAN Tetra Cell casting frame and casting stand

1.3 Specifications

Casting Stand*	Polycarbonate Pin, retaining ring, and spring Stainless steel
Casting Frames*	Polysulfone
Gray gaskets	Thermoplastic rubber (gray)
Electrode Assembly	Glass-filled polybutylene terephthalate
Electrodes	Platinum wire, 0.010 inches diameter Gasket, electrode inner core Silicone rubber (green)
Mini Tank and Lid	Polycarbonate
Sample Loading Guides**	Delrin
Combs*	Polycarbonate
Overall Size	(W x L x H, cm) 12 x 16 x 18
Precast Gel Compatibility	Ready Gel and Mini-PROTEAN precast gels (for more information, go to www.bio-rad.com/mpgels)
Voltage Limit	600 V DC and 500 W
Shipping Weight	2.0 kg

Maximum Sample Volume per Well

# Wells	Well Width	0.75 mm	1.0 mm	1.5 mm
5	12.7 mm	20 µl	105 µl	160 µl
9	5.08 mm	33 µl	44 µl	66 µl
10	5.08 mm	33 µl	44 µl	66 µl
15	3.35 mm	20 µl	26 µl	40 µl
IPG	6.2 mm	–	420 µl	730 µl
Prep/2-D				
Reference well	3.1 mm	13 µl	17 µl	30 µl
Sample well	71.1 mm	310 µl	400 µl	680 µl

Setup and Basic Operation

2.1 Gel Cassette Preparation

Hand-cast Gels

1. Glass Cassette and Casting Stand Assembly Note: All glass plates should be clean and dry.
   a. Place the casting frame upright with the pressure cams in the open position and facing forward on a flat surface.

   b. Select a spacer plate of the desired gel thickness and place a short plate on top of it (see Figure 3a).

   c. Orient the spacer plate so that the labeling is up. Slide the two glass plates into the casting frame, keeping the short plate facing the front of the frame (side with pressure cams) (see Figure 3b).

Note: Ensure that both plates are flush on a level surface and that the labels on the spacer plate are oriented correctly. Leaking may occur if the plates are misaligned or oriented incorrectly.

   d. When the glass plates are in place, engage the pressure cams to secure the glass cassette sandwich in the casting frame (see Figure 3c). Check that both plates are flush at the bottom.

   e. Place the casting frame into the casting stand by positioning the casting frame (with the locked pressure cams facing out) onto the casting gasket while engaging the spring-loaded lever of the casting stand onto the spacer plate (see Figure 3d).

Note: The gray casting stand gaskets must be clean and dry. The casting stand gaskets are made of a special thermoplastic material that swells when soaked in water, so we recommend that you do not soak the gaskets for prolonged periods prior to casting. If the gaskets do get accidentally soaked and display swelling and/or deformation, just allow them to air dry and they will regain their original shape, size and performance.

f. Repeat steps a–e for additional gels.

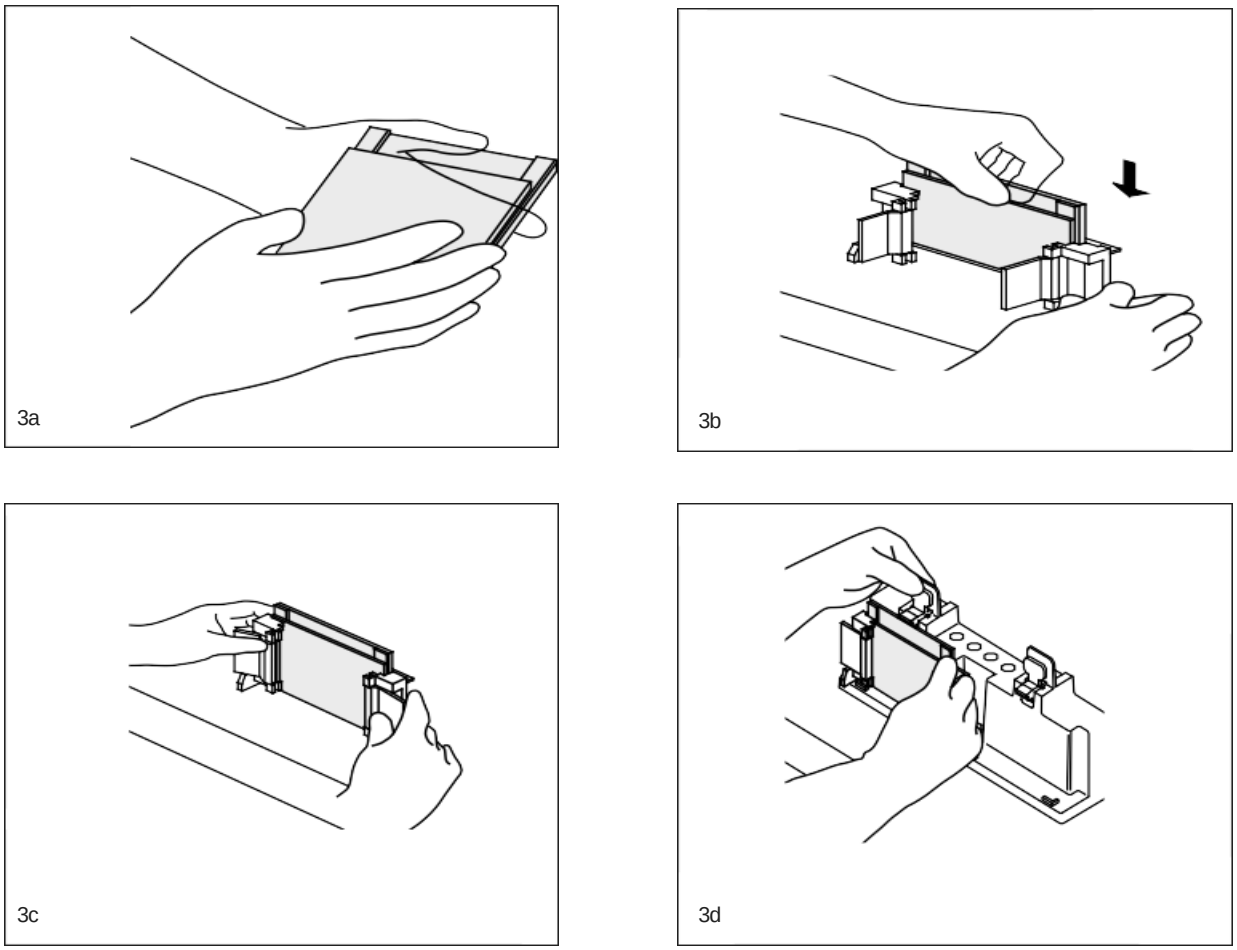

Fig. 3. Assembling the Mini-PROTEAN casting stand and frame.

2.2 Electrophoresis Module Assembly and Sample Loading Required materials:

- Clean and dry Mini-PROTEAN Tetra cell tank
- Electrophoresis module (electrode assembly module only for 1 or 2 gels; for 3 or 4 gels also use the companion running module)
- Running buffer (700 ml for 2 gels; 1000 ml for 4 gels)
- Ready Gel precast gels or hand-cast gels
- PowerPac™ Basic power supply

1. Assembly

Note: When running 2 gels only, use the electrode assembly (the one with the banana plugs), not the companion running module (the one without the banana plugs). When running 4 gels, both the electrode assembly and the companion running module must be used, for a total of 4 gels (2 gels per assembly).

a. Set the clamping frame to the open position on a clean flat surface (see Figure 4a).

b. Place the first gel sandwich or gel cassette (with the short plate facing inward) onto the gel supports; gel supports are molded into the bottom of the clamping frame assembly; there are two supports in each side of the assembly. Note that the gel will now rest at a 30° angle, tilting away from the center of the clamping frame. Please use caution when placing the first gel, making sure that the clamping frame remains balanced and does not tip over. Now, place the second gel on the other side of the clamping frame, again by resting the gel onto the supports. At this point there will be two gels resting at an angle, one on either side of the clamping frame, tilting away from the center of the frame (see Figure 4b).

Note: It is critical that gel cassettes are placed into the clamping frame with the short plate facing inward. Also, the clamping frame requires 2 gels to create a functioning assembly. If an odd number of gels (1 or 3) is being run, you must use the buffer dam (see Figure 4b).

c. Using one hand, gently pull both gels towards each other, making sure that they rest firmly and squarely against the green gaskets that are built into the clamping frame; make certain that the short plates sit just below the notch at the top of the green gasket.

d. While gently squeezing the gel sandwiches or cassettes against the green gaskets with one hand (keeping constant pressure and both gels firmly held in place), slide the green arms of the clamping frame over the gels, locking them into place. Alternatively, you may choose to pickup the entire assembly with both hands,

making sure that the
gels do not shift, and simultaneously sliding both arms of the clamping frame into place (see Figure 4c).

The arms of the clamping frame push the short plates of each gel cassette up against the notch in the green gasket, creating a leak-proof seal (check again to make certain that the short plates sit just below the notch at the top of the green gasket). At this point, the sample wells can be washedout with running buffer, and sample can be loaded (Figure 4d).

Note: If running more than 2 gels, repeat steps 1a–d with the companion running module

Important Note: Do not attempt to lock the green arms of the clamping frame, without first ensuring that the gel cassettes are perfectly aligned and stabilized against the notches on the green gaskets of the module. To prevent the gels from shifting during the locking step, firmly and evenly grip them in place against the core of the module with one hand.

Caution: When running 1 or 2 gels only, do not place the companion running Module in the tank. Doing so will cause excessive heat generation and prevent electrophoretic separation.

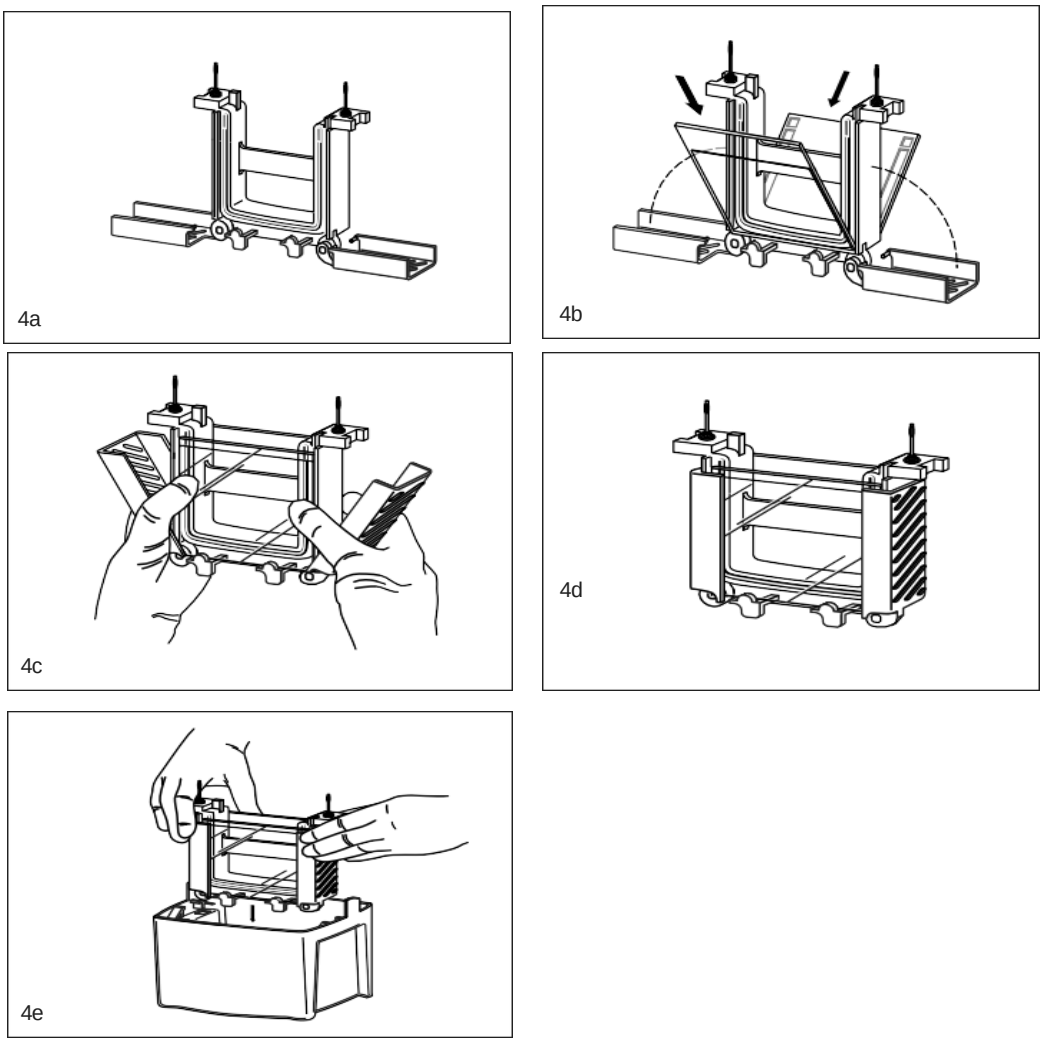

Fig. 4. Assembling the Mini-PROTEAN Tetra cell electrophoresis module.

2. Sample Loading

a. Fill the assembly (upper chamber) with buffer to just under the edge of the outer gel plate.

b. Load samples into each of the assemblies while they are sitting on a flat surface, outside of the tank.

c. Load the samples into the wells with a Hamilton syringe or a pipet using gel loading tips.

d. If using Bio-Rad's patented sample loading guide, place it between the two gels in the electrode assembly. Sample loading guides are available for 9, 10, 12, and 15-well formats.

e. Use the sample loading guide to locate the sample wells. Insert the Hamilton syringe or pipet tip into the slots of the guide and fill the corresponding wells.

Note: Load samples slowly to allow them to settle evenly on the bottom of the well. Be careful not to puncture the bottom of the well with the syringe needle or pipet.

Note: Samples may be loaded in the modules prior to placing the modules into the tank. Samples may also be loaded in the modules after the modules have been placed into the tank. Both methods will produce acceptable results. In both instances, the assembly (upper chamber) and the tank (lower chamber) should be filled with buffer according to the instructions under 2.2.2a and 2.2.3d.

3. Placement of the Electrode Assemblies in the Mini-PROTEAN Tetra Tank

Note: required total buffer volume, 700 ml for 2 gels; 1000 ml for 4 gels.

The Mini-PROTEAN Tetra tank has two positions in which to place two assemblies: the electrode assembly (back position) and the companion running module (front position).

a. Begin by placing the tank on a flat surface, with the front of the tank facing you (the front of the tank is the face that has the 2-Gels and 4-Gels line markings); when oriented properly, the red marking on the top inside edge of the tank will be on your right, and the black marking on the top inside edge of the tank will be on your left.

b. If running 2 gels only, you will be using just the electrode assembly, so place this assembly in the back position of the cell, making sure that the red (+) electrode jack matches the red marking on the top right inside edge of the tank.

c. If running 4 gels, place the electrode assembly (banana plugs) in the back position (as detailed in 2.2.3b.) and the companion running module (no banana plugs) in the
front position. Make sure that in both instances the red (+) electrode is matching with the red marking on the top inside right edge of the tank. Note that incorrect orientation will not permit proper placement of the lid.

d. Fill the tank (lower chamber) with buffer to the indicated level (550 ml for 2 gels and 680 ml for 4 gels).

4. Mini-PROTEAN Tetra Tank Assembly

a. Place the lid on the Mini-PROTEAN Tetra tank. Make sure to align the color-coded banana plugs and jacks. The correct orientation is made by matching the jacks on the
lid with the banana plugs on the electrode assembly. A stop on the lid prevents incorrect orientation. Note that the raised tabs on each side of the tank will now slide through the slots in the lid, guiding the lid to a proper close. At this point, firmly, yet gently, press down on the lid with your thumbs using even pressure, till the lid is securely and tightly positioned on the tank.

Caution: When running 1 or 2 gels only, do not place the companion running module in the tank. Doing so will cause excessive heat generation and will prevent electrophoretic separation.

5. Power Conditions

a. Insert the electrical leads into a suitable power supply with the proper polarity.

b. Apply power to the Mini-PROTEAN Tetra cell and begin electrophoresis; 200 V constant is recommended for SDS-PAGE and most native gel applications. The same voltage (200 V) is used for both 2 and 4 gels. The optimal voltage for your application may differ. Run time is approximately 35 min* at 200 V for SDS-PAGE.

* Electrophoresis time will vary between 35 and 45 min for Tris-HCl gels, depending on acrylamide percentage levels.

6. Gel Removal

a. After electrophoresis is complete, turn off the power supply and disconnect the electrical leads.

b. Remove the tank lid and carefully lift out the electrode assemblies. Pour off and discard the running buffer.

Note: Always pour off the buffer before opening the arms of the assembly, to avoid spilling the buffer.

c. Open the arms of the assembly and remove the gel cassettes.

d. Remove the gels from the gel cassette by gently separating the two plates of the gel cassette.

Note: To remove the gel from a Ready Gel cassette, first slice the tape along the sides of the Ready Gel cassette where the inner glass plate meets the outer plastic plate.

e. Remove the gel by floating it off the plate by inverting the gel and plate under fixative or transfer solution, agitating gently until the gel separates from the plate.

f. Rinse the Mini-PROTEAN Tetra cell electrode assembly, clamping frame, and mini tank with distilled, deionized water after use.

Maintenance

Mini-PROTEAN Tetra tank and lid, Rinse thoroughly with distilled water electrode assembly, companion after every use. assembly, casting stand, and frame	Rinse thoroughly with distilled water electrode after every use
Glass plates and combs	Wash with a laboratory detergent, then rinse thoroughly with distilled water. Limit submersion of spacer plates in strongly basic solutions, such as >100 mM NaOH, to less than 24 hr. Limit submersion in chromic-sulfuric acid glass cleaning solution to 2–3 hr. Prolonged submersion compromises the integrity of the adhesive. To preserve the longevity of the adhesive bond, avoid extended submersion (>5 days) in a cleaning solution made from Bio-Rad cleaning concentrate (catalog #161-0722) or other strongly basic detergents.