Mini-PROTEAN® Tetra Cell

Instruction Manual

Catalog Numbers

165-8000	165-8004
165-8001	165-8005
165-8002	165-8006
165-8003	165-8007

BIO-RAD

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Section 1
General Information

1.1 Introduction

The Mini-PROTEAN® Tetra cell runs both handcast 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 handcasting 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 form 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.

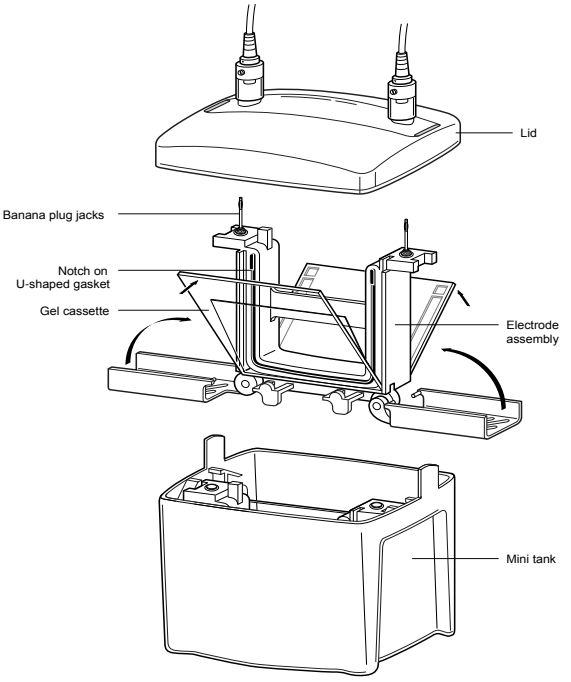

Fig. 1. Assembling the Mini-PROTEAN Tetra Cell.

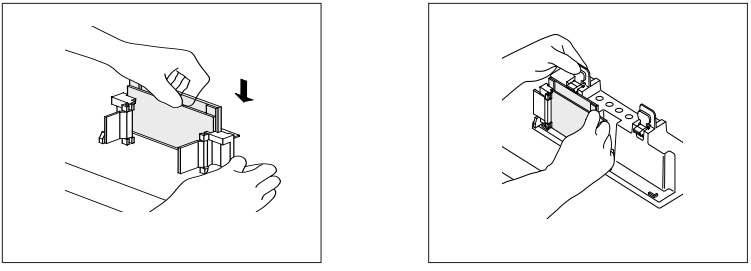

Fig. 2. Assembling the Mini-PROTEAN Tetra Cell casting frame and casting stand

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

* US patent No. 6,162,342
** US patent No. 5,656,145

Chemical Compatibility

Mini-PROTEAN Tetra cell components are not compatible with acetone or ethanol. Use of organic solvents voids all warranties. Call 1-800-4-BIORAD (US) or your local Bio-Rad representative for technical information regarding chemical compatibility of the Mini-PROTEAN Tetra cell with various laboratory reagents.
The Mini-PROTEAN are not compatible with repeated exposure to 100% TEMED. Rubbing the combs with TEMED prior to casting will destroy the structural integrity of the combs over time.

1.4 Safety

Power to the Mini-PROTEAN Tetra cell is supplied by an external DC voltage power supply (not included). The output of this power supply must be isolated from external ground to ensure that the DC voltage output floats with respect to ground. All Bio-Rad power supplies meet this important safety requirement. Regardless of the power supply used, the maximum specified operating parameters for the Mini-PROTEAN Tetra cell are as follows

- 600 V DC maximum voltage limit
- 500 W maximum power limit
- 40°C maximum ambient temperature limit

The current to the cell enters the unit through the lid assembly, which provides a safety interlock to the user. The current to the cell is broken when the lid is removed. Always turn off the power supply before removing the lid. **Do not attempt to use the cell without the safety lid.**

Important: This Bio-Rad product is designed and certified to meet IEC61010-1 and EN61010-1* 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 warranty
- Void the IEC61010-1 and EN61010-1 certifications, and
- 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 those for which it is intended or by modifications of the instrument not performed by Bio-Rad or an authorized agent.

*IEC61010-1 and EN61010-1 are internationally accepted electrical safety standards for laboratory instruments.

Section 2
Setup and Basic Operation

2.1 Gel Cassette Preparation

Handcast 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.

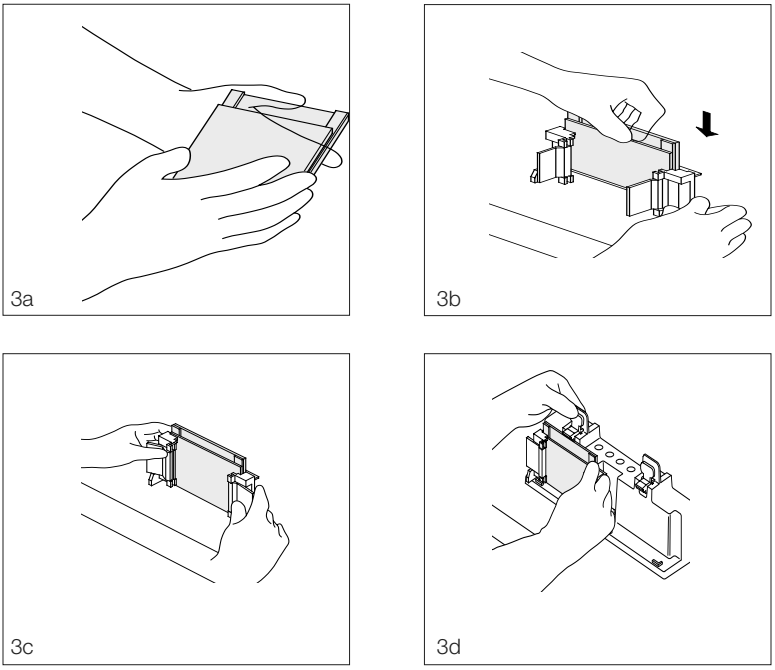

Fig. 3. Assembling the Mini-PROTEAN casting stand and frame.

2.0 Gel Casting

a. Discontinuous Polyacrylamide Gels

i. Place a comb completely into the assembled gel cassette. Mark the glass plate 1 cm below the comb teeth. This is the level to which the resolving gel is poured. Remove the comb.

ii. Prepare the resolving gel monomer solution by combining all reagents except APS and TEMED. (Refer to section 4 for gel formulations.) Degas the solution under vacuum for at least 15 min. Do not use a sink water aspirator.

iii. Add APS and TEMED to the degassed monomer solution and pour to the mark using a glass or disposable plastic pipet. Pour the solution smoothly to prevent it from mixing with air.

iv. Immediately overlay the monomer solution with water or t-amyl alcohol.

Note: If water is used, add it slowly and evenly to prevent mixing. Do not overlay with butanol or isobutanol.

v. Allow the gel to polymerize for 45 min to 1 hr. Rinse the gel surface completely with distilled water. Do not leave the alcohol overlay on the gel for more than 1 hr because it will dehydrate the top of the gel.

Note: At this point the resolving gel can be stored at room temperature overnight. Add 5 ml of 1:4 dilution of 1.5 M Tris-HCl, pH 8.8 buffer (for Laemmli system) to the resolving gel to keep it hydrated. If using another buffer system, add 5 ml 1x resolving gel buffer for storage.

vi. Prepare the stacking gel monomer solution. Combine all reagents except APS and TEMED. Degas under vacuum for at least 15 min.

vii. Dry the top of the resolving gel with filter paper before pouring the stacking gel.

viii. Add APS and TEMED to the degassed stacking gel monomer solution and pour the solution between the glass plates. Continue to pour until the top of the short plate is reached.

b. Continuous Polyacrylamide Gels

i. Prepare the monomer solution by combining all reagents except the APS and the TEMED. Degas under vacuum for 15 min (refer to section 4 for gel formulations).

ii. Add APS and TEMED to the degassed monomer solution and pour the solution between the glass plates. Continue to pour until the top of the short plate is reached.

iii. Insert the desired comb between the spacers starting at the top of the spacer plate, making sure that the tabs at the ends of each comb are guided between the spacers. Seat the comb in the gel cassette by aligning the comb ridge with the top of the short plate.

iv. Rinse the casting frame(s) and stand with distilled, deionized water after use.

Ready Gel® Precast Gels

1. Ready Gel Cassette Preparation

Note: The Mini-PROTEAN Tetra cell is guaranteed for use with Bio-Rad's Ready Gel and Mini-PROTEAN® precast gels. For more information, go to www.bio-rad.com/mpgels.

a. Remove the Ready Gel from the storage pouch.

b. Gently remove the comb and rinse the wells thoroughly with distilled water or running buffer.

c. Cut along the dotted line at the bottom of the Ready Gel cassette with a razor blade.

d. Pull the clear tape at the bottom of the Ready Gel cassette to expose the bottom edge of the gel.

e. Repeat for second Ready Gel.

Note: If only one or three gels are to be run, use the mini cell buffer dam.

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.

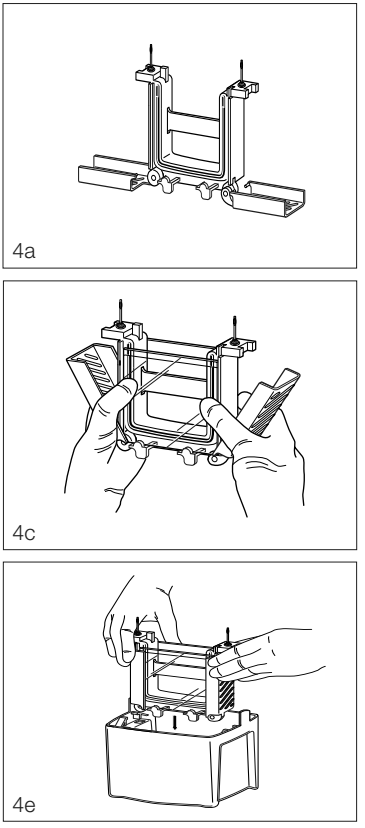

Fig. 4. Assembling the Mini-PROTEAN Tetra cell electrophoresis module.

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.

Section 3
Separation Theory and Optimization

3.1 Introduction

Polyacrylamide gel electrophoresis separates molecules in complex mixtures according to size and charge. During electrophoresis there is an intricate interaction of samples, gel matrix buffers, and electric current resulting in separate bands of individual molecules. Hence the variables that must be considered in electrophoresis are gel pore size, gel buffer systems, and the properties of the molecule of interest.

Gel Pore Size

Gel pores are created by the crosslinking of polyacrylamide with bis-acrylamide (bis) to create a network of pores. This structure allows the molecular sieving of molecules through the gel matrix. Gel pore size is a function of the acrylamide monomer concentration used (%T). By convention, polyacrylamide gels are characterized by %T, which is the weight percentage of the total monomer including the crosslinker. The %T gives an indication of the relative pore size of the gel. In general, pore size decreases with increasing %T.

%T is calculated using the following equation.

$$\%T = \frac{\text{g acrylamide + g crosslinker x 100\%}}{\text{total volume (ml)}}$$

%C is the ratio of the crosslinker to the acrylamide monomer ratio in the monomer solution. %C is calculated using the following equation.

$$\%C = \frac{\text{g crosslinker x 100\%}}{\text{g acrylamide + g crosslinker}}$$

2.67% C is traditionally used for most analytical gels.

Gels can be made as a single continuous percentage throughout the gel, or can be cast as a gradient %T through the gel. Typical compositions are from 7.5% up to 20% for single percentage gels, or gradients ranging from 4–15% to 10–20%.

The total monomer concentration for optimal separation is referred to as optimal %T. Optimal %T will vary depending on the molecular weight of the molecule of interest. Empirically the pore size providing optimum resolution for proteins is that which results in a relative mobility (Rf) value between 0.55–0.6. Rf values for specific proteins are calculated as follows.

$$Rf = \frac{\text{Distance migrated by the protein of interest}}{\text{Distance migrated by the ion front}}$$

Gel Buffer System

The buffer system determines the power requirements and affects separation. The buffer system is composed of the buffer used in the gel and the running buffer. There are continuous and discontinuous buffer systems.

Continuous Buffer Systems

In continuous buffer systems, the same buffer ions are present at constant pH in the gel and electrode reservoirs. The gel is typically made of one continuous %T and the sample is loaded directly into the part of the gel where separation will occur. The band width is determined in part by the height of the sample load, so samples should be concentrated and volumes small for best results.

Discontinuous Buffer Systems

In discontinuous buffer systems different buffer ions are present in the gel and electrode reservoirs. By using different buffers in the gel and in the electrode solutions and adding a stacking gel to the resolving gel, samples are compressed into a thin starting band and individual proteins are finely resolved and separated. Discontinuous buffer systems were devised initially for use with undenatured, or native proteins; however the most popular discontinuous system employed is the SDS-PAGE buffer system by Laemmli (1970). Formulations for this system are included in section 4.1.

3.2 SDS-PAGE (Laemmli) Buffer System

The Laemmli buffer system is a discontinuous buffer system that incorporates SDS in the buffer. In this system, proteins are denatured by heating them in buffer containing sodium dodecyl sulfate (SDS) and a thiol reducing agent such as 2-mercaptoethanol. The resultant denatured polypeptides take on a rod-like shape and a uniform charge-to-mass ratio proportional

to their molecular weights. Proteins are separated according to their molecular weights, making this system extremely useful for calculating molecular weights.

3.3 Native PAGE

Native PAGE is a technique for separating biologically active proteins. In contrast to SDS-PAGE, the mobilities of proteins in a native PAGE system depend on both size and charge. There is no single electrophoresis buffer system that will optimally purify all native proteins. Key parameters for separating proteins in a native PAGE system are isoelectric point (pI) of the protein of interest and the pH of the electrophoresis buffer.

pH and pI

The pH of the electrophoresis buffer must be within the pH range over which the protein of interest is stable and retains biological activity. In addition, the pH of the buffer must impart sufficient charge to the protein for it to move through the gel. Changes in pH will affect both the charge and size (hydrodynamic volume) of the protein of interest and will affect migration rates. For example, a buffer with a pH greater than the pI of the protein will impart a negative charge on the protein and it will migrate toward the positive electrode (anode). Conversely, a buffer with a pH lower than the pI of the protein will impart a positive charge and the protein will migrate to the negative electrode (cathode). A pH equal to the pI will result in no net charge in the protein and it will not migrate in an electric field.

Protein mobilities are best modified by the buffer's pH. Buffers with a pH closer to the pI will provide the best resolution. However run times may be lengthy. Conversely, buffers with a pH further from the pI will migrate quickly but resolution may be compromised. The choice of pH becomes a tradeoff between separation and speed.

How to Choose a Native PAGE System

1. Discontinuous Buffer Systems (Ornstein and Davis 1964)

   This discontinuous buffer system should be the first nondenaturing gel system tried. Detailed protocols are provided in section 4.2. The advantage of a discontinuous system is the use of a stacking gel to concentrate dilute protein samples. However, the stacking phenomena can also cause aggregation of some proteins and interfere with resolution. If protein aggregation occurs, a continuous buffer system should be used.

Note: The pH attained in the resolving gel of the Ornstein-Davis system approaches pH 9.5, which may be outside the range of stability for some proteins, causing denaturation. Additionally, the pI of the protein of interest may be too close to or above the Ornstein-Davis buffer pH (9.5), which may result in a very low net charge or a positive net charge that may significantly reduce or even prohibit migration to the anode. Alternative discontinuous systems can be found in an article by Chrambach and Jovin (1983).

Note: It is very desirable to know the pI of the protein of interest before selecting a buffer system.

2. Continuous Buffer Systems

A continuous buffer system will be required if discontinuous systems cannot be used due to stacking-induced protein aggregation. In a continuous system the same buffer is used in the upper and lower electrode chambers as in the gel. Since stacking does not occur, proteins migrate in bands at least as wide as the applied sample. Consequently, sample volumes should be minimized. The mobility of proteins in a continuous system is dictated by pH rather than by sieving through the polyacrylamide gel. For this reason, 6% polyacrylamide gels are recommended for most applications. For very large proteins, 4% or 5% gels may be used. McLellan describes various continuous buffer systems from pH 3.8–10.2. Detailed protocols are provided in section 4.3.

Section 4
Reagent Preparation and Stock Solutions

4.1 Volumes Required Per Gel

The volumes listed are required to completely fill a gel cassette. Amounts may be adjusted depending on the application (with or without comb, with or without stacking gel, etc.).

Gel Thickness (mm)	Volume (ml)
0.5	2.8
0.75	4.2
1.0	5.6
1.5	8.4

Note: 10 ml of monomer solution is sufficient for two stacking gels of any thickness.

4.2 SDS-PAGE (Laemmli) Buffer System Stock Solutions and Buffers

1. Acrylamide/Bis (30%T, 2.67%C)

   87.6 g acrylamide (29.2 g/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:
   Preweighed acrylamide/bis, 37.5:1 mixture (30%T, 2.67% C) (Bio-Rad catalog #161-0125, 150 g)
   30% acrylamide/bis solution, 37.5:1 mixture (30%T, 2.67% C) (Bio-Rad catalog #161-0158, 500 ml)
   (Bio-Rad catalog #161-0159, 2 x 500 ml)

2. 10% (w/v) SDS

   Dissolve 10 g SDS in 90 ml water with gentle stirring and bring to 100 ml with deionized water. Alternatively, 10% SDS solution (250 ml) can be used (Bio-Rad catalog #161-0416).

3. 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 6 N HCl. Bring total volume to 150 ml with deionized water and store at 4°C. Alternatively, 1.5 M Tris-HCl, pH 8.8 (1 L) premixed buffer can be used (Bio-Rad catalog #161-0798).

4. 0.5 M Tris-HCl, pH 6.8

   6 g Tris base
   60 ml deionized water

   Adjust to pH 6.8 with 6 N HCl. Bring total volume to 100 ml with deionized water and store at 4°C. Alternatively, 0.5 M Tris-HCl, pH 6.8 (1 L) premixed buffer can be used (Bio-Rad catalog #161-0799).

5. Sample buffer (SDS reducing buffer)

   3.55 ml deionized water
   1.25 ml 0.5 M Tris-HCl, pH 6.8
   2.5 ml glycerol
   2.0 ml 10% (w/v) SDS
   0.2 ml 0.5% (w/v) Bromophenol Blue
   9.5 ml total volume

   Store at room temperature.
   Use: Add 50 µl ß-mercaptoethanol to 950 µl sample buffer prior to use. Dilute the sample at least 1:2 with sample buffer and heat at 95°C for 4 min.

6. 10x electrode (running) buffer, pH 8.3 (makes 1 L)

   30.3 g Tris base
   144.0 g glycine
   10.0 g SDS

   Dissolve and bring total volume up to 1,000 ml with deionized water. Do not adjust pH with acid or base. Store at 4°C. If precipitation occurs, warm to room temperature before use. Alternatively, electrophoresis running buffer 10x Tris/glycine/SDS, 5 L cube (Bio-Rad catalog #161-0772) can be used.
   Use: Dilute 50 ml of 10x stock with 450 ml deionized water for each electrophoresis run. Mix thoroughly before use.

7. 10% (w/v) APS (fresh daily)

   100 mg ammonium persulfate
   Dissolve in 1 ml of deionized water.

Gel Formulations (10 ml)

1. Prepare the monomer solution by mixing all reagents except the TEMED and 10% APS. Degas the mixture for 15 min.

30 % Degassed				
Percent	DDI H_2O	Acrylamide/Bis	Gel buffer	10% w/v SDS
gel	(ml)	(ml)	(ml)	(ml)
4%	6.1	1.3	2.5	0.1
5%	5.7	1.7	2.5	0.1
6%	5.4	2.0	2.5	0.1
7%	5.1	2.3	2.5	0.1
8%	4.7	2.7	2.5	0.1
9%	4.4	3.0	2.5	0.1
10%	4.1	3.3	2.5	0.1
11%	3.7	3.7	2.5	0.1
12%	3.4	4.0	2.5	0.1
13%	3.1	4.3	2.5	0.1
14%	2.7	4.7	2.5	0.1
15%	2.4	5.0	2.5	0.1
16%	2.1	5.3	2.5	0.1
17%	1.7	5.7	2.5	0.1

* Resolving Gel Buffer – 1.5 M Tris-HCl, pH 8.8
* Stacking Gel Buffer – 0.5 M Tris-HCl, pH 6.8

2. Immediately prior to pouring the gel, add:

   For 10 ml monomer solution:

   Resolving gel: 50 µl 10% APS and 5 µl TEMED

   Stacking gel: 50 µl 10% APS and 10 µl TEMED

   Swirl gently to initiate polymerization.

   Note: Prepare any desired volume of monomer solution by using multiples of the 10 ml recipe. The volumes of APS and TEMED must be adjusted accordingly.

   Warning: The catalyst concentration is very important! Webbing and incomplete well formulation can result from inaccurate catalyst concentration.

4.3 Discontinuous Native PAGE (Ornstein-Davis) Stock Solutions and Buffers

1. Acrylamide/Bis (30%T, 2.67%C)

   87.6 g acrylamide (29.2 g/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:
   Preweighed acrylamide/bis, 37.5:1 mixture
   (Bio-Rad catalog #161-0125, 150 g)
   30% acrylamide/bis solution, 37.5:1 mixture
   (Bio-Rad catalog #161-0158, 500 ml)
   (Bio-Rad catalog #161-0159, 2 x 500 ml)

2. 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 6 N HCl. Bring total volume up to 150 ml with deionized water and store at 4°C. Alternatively, 1.5 M Tris-HCl, pH 8.8 (1 L) premixed buffer can be used (Bio-Rad catalog #161-0798).

3. 0.5 M Tris-HCl, pH 6.8

   6 g Tris base
   60 ml deionized water

   Adjust to pH 6.8 with 6 N HCl. Bring total volume up to 100 ml with deionized water and store at 4°C. Alternatively, 0.5 M Tris-HCl, pH 6.8 (1 L) premixed buffer can be used (Bio-Rad catalog #161-0799).

4. Sample Buffer

   5.55 ml deionized water
   1.25 ml 0.5 M Tris-HCl, pH 6.8
   3.0 ml glycerol
   0.2 ml 0.5% (w/v) Bromophenol Blue
   10.0 ml Total volume

   Store at room temperature.
   Use: Dilute the sample at least 1:2 with sample buffer.

5. 10x electrode (running) buffer, pH 8.3
   30.3 g Tris base (15 g/L)
   144.1 g glycine (72 g/L)

   Bring total volume up to 1,000 ml with deionized water. Do not adjust pH. Alternatively, electrophoresis running buffer 10x Tris/glycine, 1 L (Bio-Rad catalog #161-0734) can be used.

   Usage: Dilute 100 ml of 10x stock with 900 ml deionized water for each electrophoresis run.

Gel Formulations (10 ml)

1. Prepare the monomer solution by mixing all reagents except the TEMED and 10% APS. Degas the mixture for 15 min.

30 % Degassed			
Percent	**DDI H_2O**	**Acrylamide/Bis**	**Gel buffer**
gel	**(ml)**	**(ml)**	**(ml)**
4%	6.2	1.3	2.5
5%	5.8	1.7	2.5
6%	5.5	2.0	2.5
7%	5.2	2.3	2.5
8%	4.8	2.7	2.5
9%	4.5	3.0	2.5
10%	4.2	3.3	2.5

*Resolving Gel Buffer – 1.5 M Tris-HCl, pH 8.8
*Stacking Gel Buffer – 0.5 M Tris-HCl, pH

2. Immediately prior to pouring the gel, add:

   50 ml APS and TEMED (5 µl for resolving gels; 10 µl TEMED for stacking gels)
   Swirl gently to initiate polymerization.

   Note: Prepare any desired volume of monomer solution by using multiples of the 10 ml recipe. The volumes of APS and TEMED must be adjusted accordingly.

4.4 Continuous Native PAGE Stock Solutions and Buffers

1. Acrylamide/Bis (30%T, 2.67%C)

   87.6 g acrylamide (29.2 g/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:
   Preweighed acrylamide/bis, 37.5:1 mixture
   (Bio-Rad catalog #161-0125, 150 g)
   30% acrylamide/bis solution, 37.5:1 mixture
   (Bio-Rad catalog #161-0158, 500 ml)
   (Bio-Rad catalog #161-0159, 2 x 500 ml)

2. Sample Buffer

   1.0 ml electrophoresis buffer
   3.0 ml glycerol
   0.2 ml 0.5% Bromophenol Blue
   5.8 ml deionized water
   10.0 ml total volume

3. Continuous Buffers (McLellan)

   McLellan describes various continuous buffer systems from pH 3.8 to pH 10.2. Use the table on the page to prepare 5x continuous non-denaturing PAGE electrophoresis buffers. Add both the acidic and basic component to 1 L of water. Do not adjust the pH. If the final pH is outside the listed range discard the buffer and remake.

Basic pH	Acidic Component	5x Solution	Component	5x Solution
3.8	ß-Alanine	13.36 g/L	Lactic acid	7.45 ml/L
	(MW 89.09)		85% solution	
4.4	ß-Alanine	35.64 g/L	Acetic acid	11.5 ml/L
	(MW 89.09)		17.4 M	
4.8	GABA	41.24 g/L	Acetic acid	5.75 ml/L
	(MW 103.1)		17.4 M	
6.1	Histidine	23.28 g/L	MES	29.5 g/L
	(MW 155.2)		(MW 195.2)	
6.6	Imidazole	19.94 g/L	MOPS	31.4 g/L
	(MW 68.08)		(MW 209.3)	
7.4	Tris	14.64 g/L	HEPES	41.7 g/L
	(MW 121.14)		(MW 238.33)	
8.1	Tris	19.38 g/L	EPPS	37.85 g/L
	(MW 121.14)		(MW 252.2)	
8.7	Tris	30.28 g/L	Boric acid	7.73 g/L
	(MW 121.14)		9MW 61.83)	
9.4	Tris	36.34 g/L	CAPS	44.26 g/L
	(MW 121.14)		(MW 221.3)	
1.0	Ammonia	12.5 ml/L	CAPS	22.13 g/L
	(14.8 M)		(MW 221.3)	

Dilute 200 ml of 5x buffer with 800 ml deionized water to prepare 1x electrophoresis buffer. The final concentrations of buffer components will be.

pH	Basic component	Acidic component
3.8	30 mM ß-Alanine	20 mM Lactic acid
4.4	80 mM ß-Alanine	40 mM Acetic acid
4.8	80 mM GABA	20 mM Acetic Acid
6.1	30 mM Histidine	30 mM MES
6.6	25 mM Histidine	30 mM MOPS
7.4	43 mM Histidine	35 mM HEPES
8.1	32 mM Tris 30 mM EPPS	
8.7	50 mM Tris 25 mM Boric acid	
9.4	60 mM Tris 40 mM CHAPS	
10.2	37 mM Ammonia	20 mM CAPS

Gel Formulations (10 ml)

1. Prepare the monomer solution by mixing all reagents except the TEMED and 10% APS. Degas the mixture for 15 minutes.

30 % Degassed			
Percent	**DDI H_2O**	**Acrylamide/Bis**	**Gel buffer**
gel	**(ml)**	**(ml)**	**(ml)**
4%	6.7	1.3	2.0
5%	6.3	1.7	2.0
6%	6.05	2.0	2.0

Note: Prepare any desired volume of monomer solution by using multiples of the 10 ml recipe.

2. Immediately prior to pouring the gel, for 10 ml monomer solution add:

   50 µl 10% APS
   10 µl TEMED
   Swirl gently to initiate polymerization.

   Note: Below pH 6, TEMED becomes a less effective catalyst. Increase the concentration of TEMED 5-fold to polymerize gels with a pH range between 4 and 6.

Section 5
References

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage t4. Nature 227, 680-685.

Ornstein L (1964). Disc electrophoresis. I. Background and theory. Ann N Y Acad Sci 121, 321-349.

Chrambach (1983). A and Jovin, T M, Electrophoresis, 4, 190–204

McLellan T (1982). Electrophoresis buffers for polyacrylamide gels at various ph. Anal Biochem 126, 94-99.

Section 6
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 cleaning solution made from Bio-Rad cleaning concentrate (catalog #161-0722) or other strongly basic detergents.

Section 7
Troubleshooting Guide

Problem	Cause	Solution
Smile effect – band pattern curves upward at both sides of the gel	Center of the gel running hotter than either end	Buffer not mixed well or buffer in upper chamber too concentrated. Remake buffer, ensuring thorough mixing, especially when diluting 5x or 10x stock
	Power conditions excessive	Decrease the power setting from 200 V to 150 V or fill lower chamber to within 1 cm of top of short plate
Vertical streaking of protein	Sample overloaded	Dilute sample, selectively remove predominant protein in sample, or reduce the voltage about 25% to minimize streaking
	Sample precipitation	Centrifuge sample before addition of SDS sample buffer, or decrease %T of the gel*
		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
Lateral band spreading	Diffusion of the wells prior to turning on the current	Minimize the time between sample application and turning on the power startup
	Ionic strength of the sample lower than that of the gel	Use same buffer in sample as in the gel or the stacking gel
Skewed or distorted band	Poor polymerization around wells	Degas stacking gel solution completely prior to casting; increase ammonium persulfate and TEMED concentrations by 25%, for stacking gel or low %T, leave APS the same and double the TEMED concentration
	Salts in sample	Remove the salts by dialysis, desalting, column, Micro Bio-Spin™ columns, etc.
	Uneven gel interface	Descrease the polymerization rate. Overlay gels very carefully

Troubleshooting Guide (cont.)

Problem	Cause	Solution
Lanes constricted at the bottom of the gel	Ionic strength of sample higher than the surrounding gel	Desalt sample and neighboring samples
Run taking unusually long	Running buffer too concentrated Excessive salt in sample	Check buffer protocol, dilute if necessary Desalt sample
Run too fast	Running or reservoir buffer too dilute Voltage too high	Check buffer protocol, dilute if necessary Decrease voltage by 25–58%
Doublets observed where single protein species is expected (SDS-PAGE)	A portion of the protein may have been reoxidized during the run or may not have been fully reduced prior to the run	Prepare fresh sample buffer solution if over 30 days old; increase concentration in the sample buffer; sustitute DTT for BME
Fewer bands than expected and one heavy band at the dry front	Protein(s) migrating at the dye front Protein degradation	Increase the %T of the resolving gel* Use protease inhibitors, e.g., PMSF, etc.
Upper buffer chnamber leaks	Upper buffer chamber overfilled Improper assembly	Keep buffer level below the top of the spacer plate Be sure U-shaped electrode core gasket is clean, free of cuts, and lubricated with buffer Be sure short plate is under the notch on the gasket, not on top of it
Leaking during hand casting	Chipped glass plates Spacer plate and short plate not level Csating stand gasket is dirty, flawed, or worn out.	Ensure glass plates are free of flaws Ensure plates are aligned correctly Wash the gasket if it is dirty, replace casting stand gaskets if flawed or worn out

Troubleshooting Guide (cont.)

Problem	Cause	Solution
Poor end well formation	Incorrect catalyst formation	Prepare fresh catalyst solution, or increase the catalyst concentration of the stacking gel to 0.06% APS and 0.12% TEMED
	Monomer solution not degassed. Oxygen inhibits polymerization	Degas monomer solution immediately prior to casting the stacking gel
Webbing/excess acrylamide behind the comb	Incorrect catalyst concentration	Prepare fresh catalyst solution, or increase the catalyst concentration of the stacking gel to 0.06% APS and 0.12% TEMED
The pressure cams on the casting frame are difficult to close or make noise when closed	Powder residue has built up at the pivot of the pressure cams	Rinse or wipe off the powder residue before each use

*Polyacrylamide gels are described by reference to two characteristics:

1. The total monomer concentration, (%T) and
2. The crosslinking monomer concentration (%C).

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

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

Section 8
Product Information and Accessories

Mini PROTEAN Tetra Systems

Catalog Number	Descriptions
165-8000	**Mini-PROTEAN Tetra Cell**, 10 well, 0.75 mm thickness, complete system includes 5 combs, 5 sets of glass plates, 2 casting stands, casting clamp assembly, sample loading guide, electrode assembly, companion running module, tank, lid with power cables, mini cell buffer dam
165-8001	**Mini-PROTEAN Tetra Cell,** 10 well, 1.0 mm thickness, complete system, includes 5 combs, 5 sets of glass plates, 2 casting stands, casting clamp assembly, sample loading guide, electrode assembly, companion running module, tank, lid with power cables, mini cell buffer dam
165-8002*	**Mini-PROTEAN Tetra Cell,** 10 well, 0.75 mm thickness; 2-gel system includes 5 combs, 5 sets of glass plates, casting stand, 2 casting frames, sample loading guide, electrode assembly, tank, lid with power cables, mini cell buffer dam
165-8003*	**Mini-PROTEAN Tetra Cell,** 10 well, 1.0 mm thickness; 2-gel system includes 5 combs, 5 sets of glass plates, casting stand, 2 casting frames, sample loading guide, electrode assembly, tank, lid with power cables, mini cell buffer dam
165-8004	**Mini-PROTEAN Tetra Cell** for Ready Gel® precast gels, electrode assembly, companion running module, clamping frame, tank, lid with power cables, mini cell buffer dam
165-8005*	**Mini-PROTEAN Tetra Cell for Mini Precast Gels,** 2-gel system includes electrode assembly, clamping frame, tank, lid with power cables, mini cell buffer dam
165-8006	**Mini-PROTEAN Tetra Cell,** 10 well, 1.5 mm thickness; 4-gel system includes 5 combs, 5 sets of glass plates, 2 casting stands, 4 casting frames, sample loading guide, electrode assembly, companion running module, tank, lid with power cables, mini cell buffer dam
165-8007*	**Mini-PROTEAN Tetra Cell,** 10 well, 1.5 mm thickness; 2-gel system includes 5 combs, 5 sets of glass plates, casting stand, 2 casting frames, sample loading guide, electrode assembly, tank, lid with power cables, mini cell buffer dam
165-8025	**Mini-PROTEAN Tetra Cell and PowerPac Basic Power Supply,** includes 165-8001 and 164-5050
165-8026	**Mini-PROTEAN Tetra Cell and PowerPac Universal Power Supply,** includes 165-8001 and 164-5070

Catalog Number	Descriptions
165-8027	**Mini-PROTEAN Tetra Cell and PowerPac HC Power Supply,** includes 165-8001 and 164-5052
165-8028	**Mini-PROTEAN Tetra Cell and PowerPac HV Power Supply,** includes 165-8001 and 164-5056
165-8029	**Mini-PROTEAN Tetra Cell and Mini Trans-Blot Module,** includes 165-8001 and 170-3935
165-8030	**Mini-PROTEAN Tetra Cell (for Ready Gel Precast Gels) and Mini Trans-Blot Module,** includes 165-8004 and 170-3935
165-8033	**Mini-PROTEAN Tetra Cell, Mini Trans-Blot Module, and PowerPac Basic Power Supply,** includes 165-8001, 170-3935, and 164-5050
165-8034	**Mini-PROTEAN Tetra Cell (for Ready Gel Precast Gels), Mini Trans-Blot Module, and PowerPac Basic Power Supply**, includes 165-8004, 170-3935, and 164-5050
165-8035	**Mini-PROTEAN Tetra Cell,** Mini Trans-Blot Module, and PowerPac HC Power Supply, includes 165-8001, 170-3935, and 164-5052
165-8036	**Mini-PROTEAN Tetra Cell** (for Ready Gel precast gels), Mini Trans-Blot Module, and PowerPac HC Power Supply, includes 165-8004, 170-3935, and 164-5052

***The 2-gel systems do not include the companion running module.**

Casting Modules

Each casting module includes 2 combs, 5 sets of glass plates, 2 casting stands, 4 casting frames, and the appropriate sample loading buffer.

	0.75 mm Spacer	1.0 mm Spacer	1.5 mm Spacer
5–well	165-8008	165-8013	165-8019
9–well	165-8009	165-8014	165-8020
10–well	165-8010	165-8015	165-8021
15–well	165-8011	165-8016	165-8022
Prep/2–D well	165-8012	165-8017	165-8023
IPG well	N/A	165-8018	165-8024

Handcast Gel Accesories and Replacement Parts

Catalog Number	Descriptions
165-3303	**Mini-PROTEAN Casting Stand**
165-3304	**Mini-PROTEAN Casting Frame**
165-3305	**Mini-PROTEAN Casting Stand Gaskets,** 2
165-3308	**Short Plates,** 5
165-3310	**Spacer Plates with 0.75 mm Internal Spacers,** 5
165-3311	**Spacer Plates with 1.0 mm Internal Spacers,** 5
165-3312	**Spacer Plates with 1.5 mm Internal Spacers,** 5

Other Replacements Parts

Catalog Number	Descriptions
165-8037	**Mini-PROTEAN Tetra Electrode Assembly**
165-8038	**Mini-PROTEAN Tetra Companion Running Module**
165-8039	**Buffer Tank,** replacement
165-8040	**Buffer Tank and Lid,** replacement
165-8041	**Cell Lid with Power Cables**
165-3201	**Sample Loading Guide**, 9 well (red)
165-3146	**Sample Loading Guide,** 10 well (yellow)
165-3203	**Sample Loading Guide,** 12 well (green)
165-3132	**Sample Loading Guide,** 15 well (blue)
165-3130	**Mini Cell Buffer Dams,** 2
165-3149	**Replacement Gaskets for Electrophoresis Assembly,** green, 2
161-0990	**Empty Ready Gel Cassettes,** 10

Combs

	0.75 mm	1.0 mm	1.5 mm
5–well	165-3352	165-3357	165-3363
9–well	165-3353	165-3358	165-3364
10–well	165-3354	165-3359	165-3365
15–well	165-3355	165-3360	165-3366
Prep/2–D well	165-3356	165-3361	165-3367
IPG well	N/A	165-3362	165-3368

Section 9
Warranty Information

The Mini-PROTEAN Tetra cell is warranted for one year against defects in materials and workmanship. If any defects should occur during this warranty period, Bio-Rad Laboratories will replace the defective parts without charge. However, the following defects are specifically excluded.

1. Defects caused by improper operation
2. Repairs or modifications done by anyone other than Bio-Rad Laboratories or their authorized agent
3. Damage caused by accidental misuse
4. Damage caused by disaster
5. Common replacement parts including platinum wire, the rubber gaskets, and glass plates
6. Damage caused by the use of organic solvents

For inquiries or requests for repair service, contact your local Bio-Rad office.

Warranty Information

Model_____

Catalog number_____

Date of delivery_____

Serial number_____

Invoice number_____

Purchase order number_____

* US Patent No. 6,162,342

** US patent No. 5,656,145

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