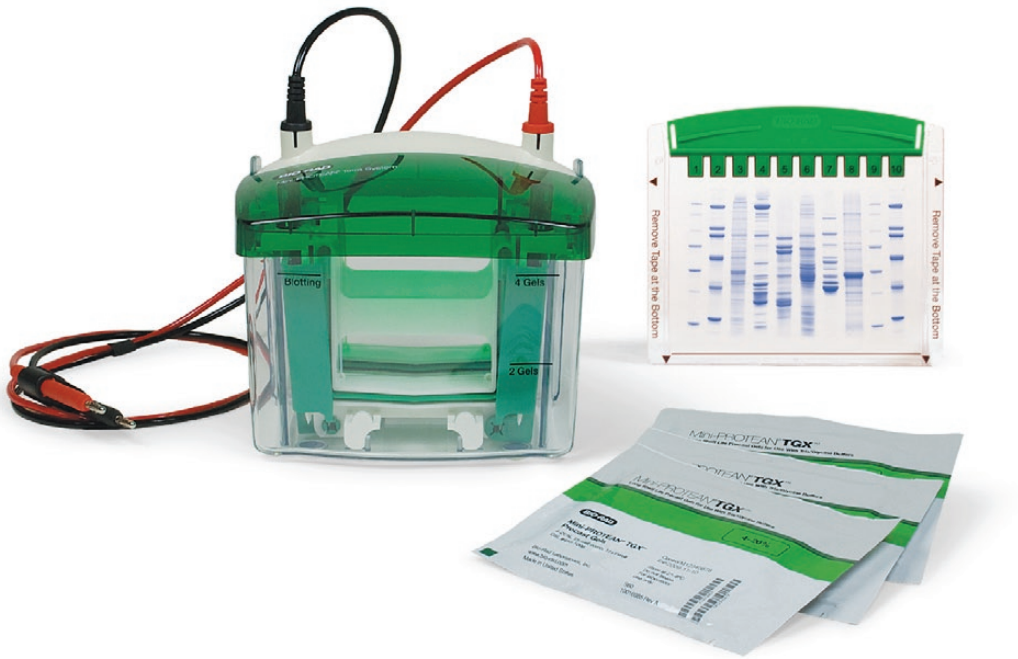
Mini-PROTEAN® Precast Gels

Instruction Manual and Application Guide



BIO-RAD

Bio-Rad Technical Support

For help and technical advice, please contact the Bio-Rad Technical Support department. In the United States, the Technical Support department is open Monday–Friday, 5:00 AM–5:00 PM, Pacific Time.

Phone: 1-800-424-6723

Fax: 1-510-741-5802

Email: LSG_TechServ_US@bio-rad.com (for U.S. and international customers)

Online technical support and worldwide contact information are available at www.consult.bio-rad.com.

Legal Notices

Contents

Chapter 1: Mini-PROTEAN® Precast Gels 1
1.1 Introduction 1
1.2 Gel Formulations 2
1.3 Comb Configurations 2
1.4 Specifications 2
1.5 Storage Conditions 2
1.6 Important Notes 3

Chapter 2: Setup and Basic Operation 4
2.1 Workflow Overview 4
2.2 Required Materials 5
2.3 Setting Up and Running Mini-PROTEAN Gels in the Mini-PROTEAN® Tetra Cell 5
2.4 Removing the Gel 7

Chapter 3: SDS-PAGE 8
3.1 Introduction 8
3.2 Mini-PROTEAN® TGX™ Gels 8
3.3 SDS-PAGE Buffers 9
3.4 Sample Preparation 10
3.5 Running Conditions 10

Chapter 4: Native PAGE 11
4.1 Introduction 11
4.2 Mini-PROTEAN TGX Gels 11
4.3 Native PAGE Buffers 11
4.4 Sample Preparation 12
4.5 Running Conditions 12

Chapter 5: 2-D Electrophoresis 13
5.1 Introduction 13
5.2 Equilibration 13
5.3 Agarose Overlay 13
5.4 Second-Dimension Electrophoresis 13

Chapter 6: Peptide Analysis 14
6.1 Introduction 14
6.2 Mini-PROTEAN Tris-Tricine Gels 14
6.2.1 Gel Composition 14
6.2.2 Gel Selection Guide 14
6.3 Peptide Analysis Buffers 15
6.4 Sample Preparation 15
6.5 Running Conditions 15

Chapter 7: Nondenaturing Nucleic Acid PAGE 16
7.1 Introduction 16
7.2 Mini-PROTEAN TBE Gels 16
7.2.1 Gel Composition 16
7.2.2 Gel Selection Guide 16
7.3 Nondenaturing Nucleic Acid PAGE Buffers 17
7.4 Sample Preparation 17
7.5 Running Conditions 17

Chapter 8: Denaturing Nucleic Acid PAGE 18
8.1 Introduction 18
8.2 Mini-PROTEAN TBE-Urea Gels 18
8.2.1 Gel Composition 18
8.2.2 Gel Selection Guide 18
8.3 Denaturing Nucleic Acid PAGE Buffers 19
8.4 Sample Preparation 19
8.5 Running Conditions 19

Chapter 9: Detection 20
9.1 SDS-PAGE and Native PAGE Detection 20
9.2 Peptide Gel Staining 21
9.3 TBE Gel Staining 21
9.4 TBE-Urea Gel Staining 21

Chapter 10: Blotting 22
10.1 Introduction 22
10.2 Transfer 22
10.2.1 Transfer Buffers 22
10.2.2 Wet Transfer Using the Mini Trans-Blot® Module 22
10.2.3 Semi-Dry Transfer Using the Trans-Blot® SD Cell 23
10.3 Total Protein Blot Stains 24
10.4 Immunodetection 24

Chapter 11: Troubleshooting 25

Appendix A: Quick Start Guides. 27
SDS-PAGE (Mini-PROTEAN TGX Gels) 28
Native PAGE (Mini-PROTEAN TGX Gels) 29
Peptide Analysis (Mini-PROTEAN Tris-Tricine Gels) 30
Nondenaturing Nucleic Acid PAGE (Mini-PROTEAN TBE Gels) 31
Denaturing Nucleic Acid PAGE (Mini-PROTEAN TBE-Urea Gels) 32
Appendix B: Buffers 33
Running Buffers 33
Sample Buffers 34
Buffer Components 35
Blotting Buffers 35
Appendix C: Related Literature 36
Appendix D: Ordering Information 37

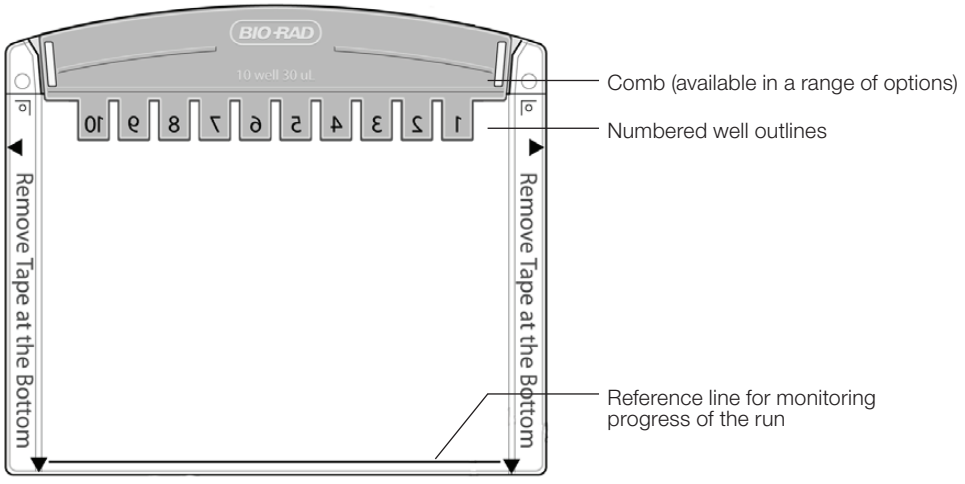
1 Mini-PROTEAN® Precast Gels

1.1 Introduction

Mini-PROTEAN precast gels are 7.2 cm x 8.6 cm gels designed for performing polyacrylamide gel electrophoresis (PAGE) with the Mini-PROTEAN family of vertical electrophoresis cells, which includes the Mini-PROTEAN® Tetra and Mini-PROTEAN® 3 Dodeca™ cells and the discontinued Mini-PROTEAN II and Mini-PROTEAN 3 cells. The Mini Trans-Blot and Trans-Blot SD blotting cells and precut membrane sandwiches are also available for blotting applications with these gels.

Features of Mini-PROTEAN precast gels include:

- Outlined and numbered well that simplify sample loading and identification
- Capacity for up to 15 samples per gel
- Bottom-open cassette design for easy gel handling and blotting setup
- Easy-to-open cassette for faster downstream processing
- Reference line at the bottom of the cassette indicates where the run should stop (for optimum resolution across the separation range)
- Excellent staining quality and transfer efficiency
- No gel foot to remove prior to blotting



1.2 Gel Formulations

Mini-PROTEAN precast gels are composed of polyacrylamide with a bisacrylamide crosslinker, and they are available in a range of formulations (Table 1.1) and in a selection of single percentages and gradients.

Table 1.1. Mini-PROTEAN precast gel formulations.

Application	Gel Formulation	Sample Buffer	Running Buffer
SDS-PAGE	Mini-PROTEAN TGX™	Laemmli	Tris/glycine/SDS
Native PAGE	Mini-PROTEAN TGX	Native	Tris/glycine
Peptide analysis	Mini-PROTEAN Tris-Tricine	Tricine	Tris/Tricine/SDS
dsDNA separation	Mini-PROTEAN TBE	Nucleic acid	Tris/boric acid/EDTA (TBE)
ssDNA and RNA separation	Mini-PROTEAN TBE-urea	TBE-urea	TBE

1.3 Comb Configurations

Comb Type	Well Volume
10-well	50 µl
10-well	30 µl
15-well	15 µl
IPG/prep	7 cm ReadyStrip™ IPG strip (450 µl)

1.4 Specifications

Gel material	Polyacrylamide
Gel dimensions	7.2 x 8.6 cm
Gel thickness	1.0 mm
Resolving gel height	6.2 cm (5.6 cm for 50 µl well)
Cassette dimensions	8.5 x 10 cm
Cassette material	Styrene copolymer
Comb material	Polycarbonate
Running buffer	750 ml for 2 gels, 1,000 ml for 4 gels (Mini-PROTEAN Tetra cell) 325 ml for 1–2 gels (Mini-PROTEAN II or Mini-PROTEAN 3 cell)

1.5 Storage Conditions

Table 1.2. Storage conditions for Mini-PROTEAN precast gels. Store gels flat. Shelf life is from date of manufacture; expiration dates are printed on the cassettes.

Storage Temperature	Gel Formulation	Shelf Life
2–8°C	Mini-PROTEAN TGX	12 months
	Mini-PROTEAN Tris-Tricine	12 weeks
	Mini-PROTEAN TBE	12 weeks
	Mini-PROTEAN TBE-urea	8 weeks

1.6 Important Notes

Use each Mini-PROTEAN precast gel as soon as possible after removing it from the storage pouch.

Improper storage of Mini-PROTEAN precast gels can produce artifacts. Store gels flat and at 2–8°C. Avoid freezing or prolonged storage above 8°C. If your gels have been stored improperly, discard them.

Do not run more than one gel type in the same apparatus at the same time. Different gel percentages and formulations have different conductivities and different run times.

With the Mini-PROTEAN Tetra cell:

- When running 1–2 gels:

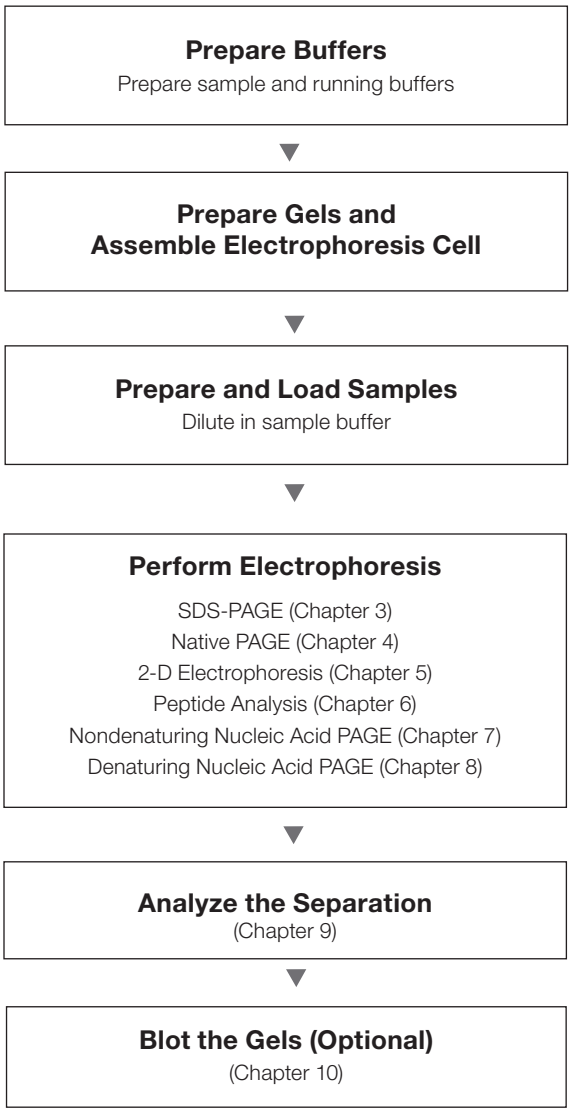
  Use the electrode assembly (with banana plugs), not the companion running module (without banana plugs)

  Do not place the companion running module in the tank. Doing so generates excessive heat and degrades the quality of the electrophoretic separation

- When running 3–4 gels, use both the electrode assembly and companion running module
- When using voltages >200 V, fill the lower buffer chamber to the 4 gel (800 ml) mark

2 Setup and Basic Operation

2.1 Workflow Overview

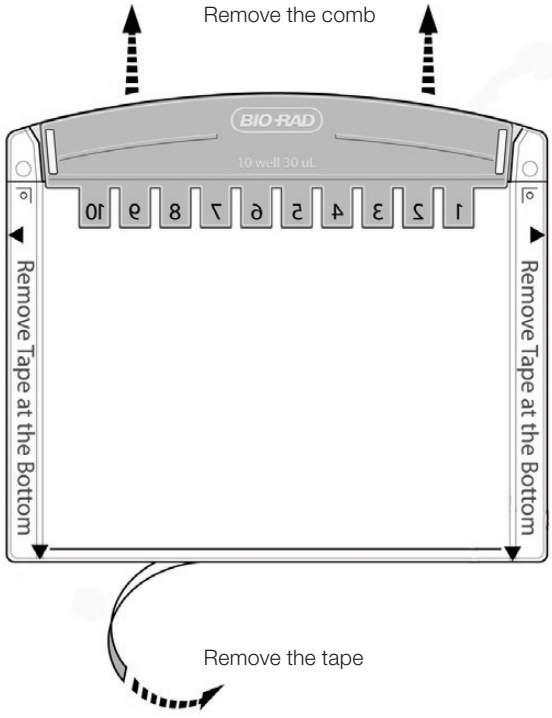


2.2 Required Materials

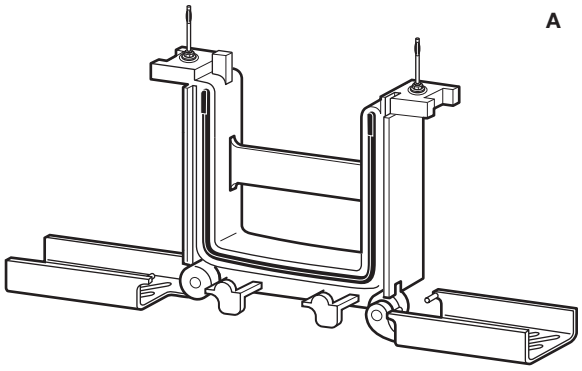
- Mini-PROTEAN® precast gels
- Mini-PROTEAN® Tetra cell (or Mini-PROTEAN® 3 Dodeca™, Mini-PROTEAN II or Mini-PROTEAN 3 cell)
- PowerPac™ Basic or PowerPac HC power supply (or equivalent); PowerPac HV or PowerPac Universal required for high-voltage applications (>300 V)
- Sample buffer
- Running buffer (750 ml for 1–2 gels; 1,000 ml for 3–4 gels)
- Opening lever (catalog #456-0000)

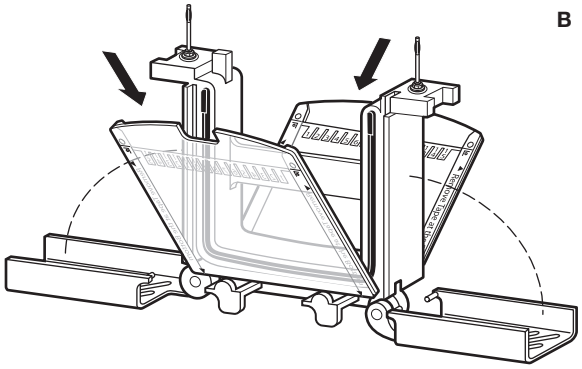
2.3 Setting Up and Running Mini-PROTEAN Gels in the Mini-PROTEAN Tetra Cell

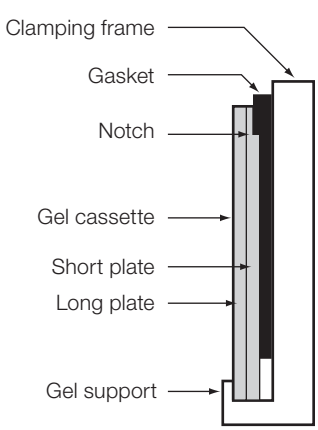
1. Remove the gels from the storage pouch and prepare them for assembly:
   a. Remove the comb: Place both thumbs on the ridges of the comb, and remove the comb by pushing upward in one smooth, continuous motion.
   b. Remove the tape: Pull gently to remove the green tape from the bottom of the cassette. If necessary, use the opening key or comb to help remove the tape at the corners.
   c. Rinse the wells: Use a syringe, wash bottle, or disposable transfer pipet to rinse the wells with running buffer. Straighten the sides of the wells, if necessary.

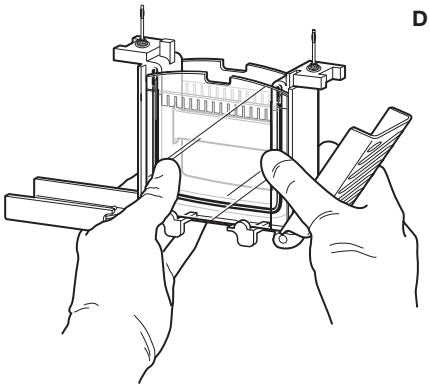


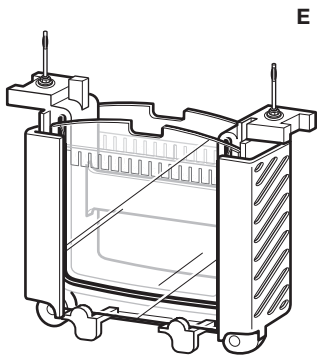
2. Set the electrode assembly to the open position on a clean, flat surface (**A**).
3. Place the gel cassettes into the electrode assembly. Two cassettes are required to create a functioning assembly; when using 1 or 3 gels, use the buffer dam (included with the cell) to complete the assembly.
   a. Place the first cassette with the short plate facing inward and so the gel rests at a 30° angle away from the center of the electrode assembly. Make sure the electrode assembly remains balanced and does not tip over.
   b. Place the second gel or buffer dam on the other side of the electrode assembly, again by resting the gel on the supports. The gels rest at 30° angles, one on either side of the electrode assembly, tilting away from the center of the frame (**B**).
4. Gently push both gels toward each other, making sure that they rest firmly and squarely against the green gasket that is built into the electrode assembly. Align the short plates to ensure the edge sits just below the notch at the top of the green gasket (**C**).
5. While gently squeezing the gel cassettes (or cassette and buffer dam) against the green gaskets (maintaining constant pressure and with both gels in place), slide the green arms of the clamping frame one at a time over the gels, locking them into place (**D**,**E**).
6. The wing clamps of the electrode assembly lift each gel cassette up against the notch in the green gasket, forming a seal. Check again that the short plates sit just below the notch at the top of the green gasket (**C**).



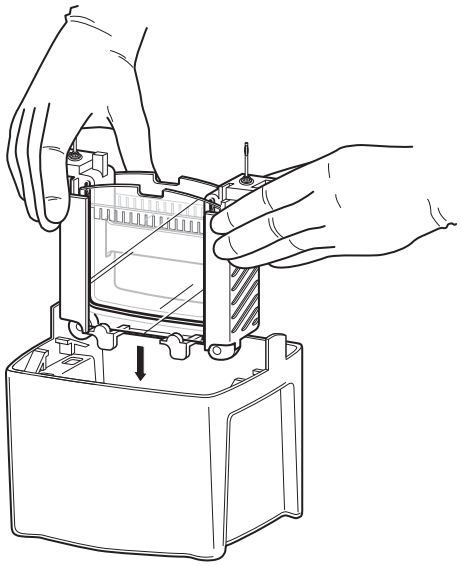








F

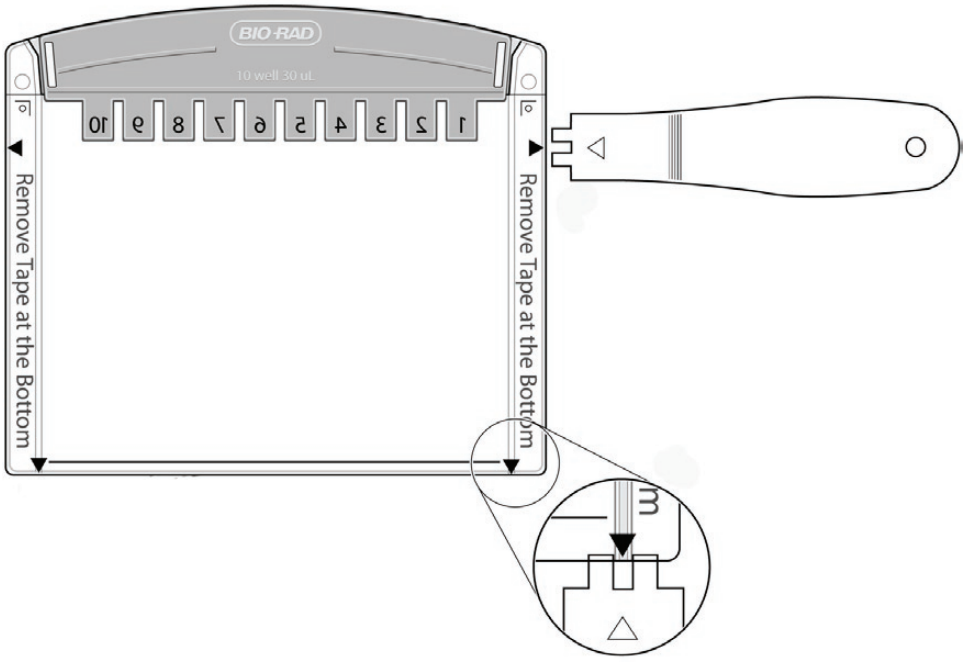


If running more than 2 gels, repeat steps 2–6 with the companion running module.

7. Place the electrophoresis module into the tank (**F**) and fill the buffer chambers with 1x running buffer:
   - 200 ml in the inner buffer chamber
   - 550 ml (1–2 gels) or 800 ml (3–4 gels, or >200 V) in the outer buffer chamber
8. Wash the sample wells with running buffer (if this was not done earlier).
9. Load samples and run the gels using the running conditions appropriate to your application. Stop the run when the dye front reaches the reference line imprinted on the bottoms of the cassettes.

2.4 Removing the Gel

1. After electrophoresis is complete, turn off the power supply and disconnect the electrical leads.
2. Remove the lid from the tank and remove the gels from the cell. Pour off and discard the running buffer.
3. To open the cassette, align the arrow on the opening lever with the arrows marked on the cassette and insert the lever between the cassette plates at all four locations. Apply downward pressure to break each seal. Do not twist the lever.
4. Pull the two plates apart from the top of the cassette, and gently remove the gel.



3 SDS-PAGE

3.1 Introduction

Mini-PROTEAN® TGX™ (**T**ris-**G**lycine e**X**tended shelf life) gels provide a versatile system for separating proteins by either molecular weight (SDS-PAGE) or mass-to-charge ratio (native PAGE). (See Chapter 4 for native PAGE applications and protocols.) This versatility is possible because the gels are made without SDS; this allows the sample buffer and running buffer to determine the separation mechanism.

SDS-PAGE relies on a discontinuous buffer system. Two ions differing in electrophoretic mobility (glycinate and chloride) form a moving boundary when voltage is applied. Proteins have an intermediate mobility that causes them to concentrate, or stack, into a narrow zone at the beginning of electrophoresis. As that zone moves through the gel, the sieving effect of the polyacrylamide gel matrix causes proteins of different molecular weighs to move at different rates. This stacking effect is responsible for the high resolving power of SDS-PAGE: the sample is loaded in a relatively broad zone, and the moving boundary concentrates the proteins into sharp bands prior to separation.

Protein samples for SDS-PAGE are prepared using SDS and a thiol reducing agent, usually β-mercaptoethanol or dithiothreitol (DTT). SDS forms complexes with proteins, giving them a rodlike shape and similar mass-to-charge ratio. The reducing agent disrupts disulfide bonds between and within proteins, allowing complete denaturation and dissociation. Heat treatment in the presence of SDS and reducing agent effectively eliminates the effects of native charge and higher order structure on electrophoretic mobility, so the migration distance depends primarily on molecular weight.

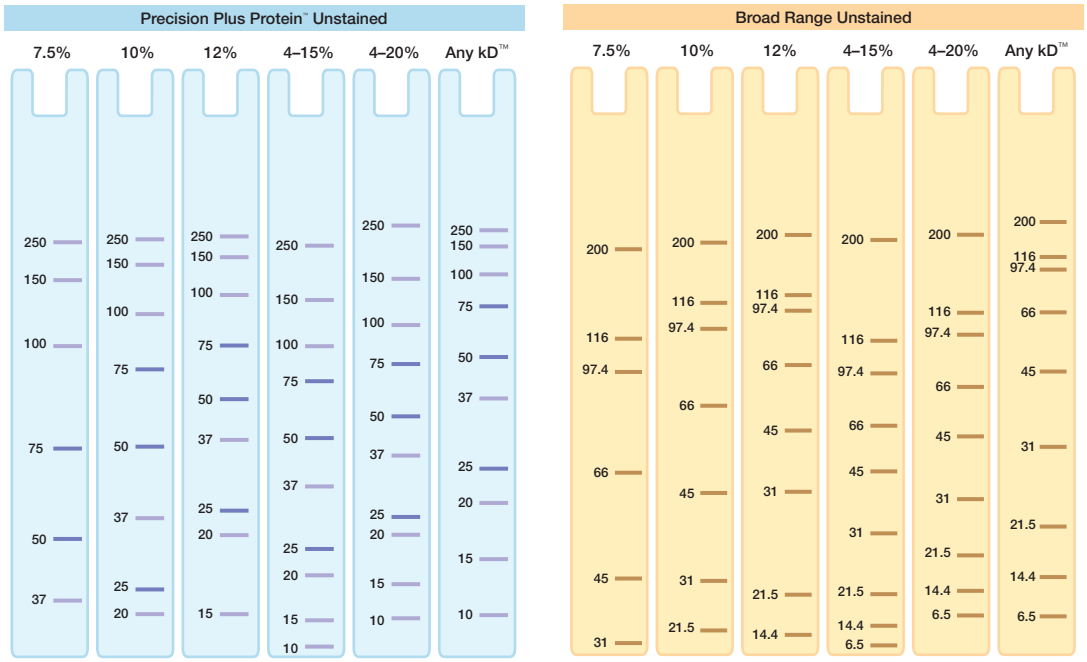
Molecular weight is estimated by plotting the logarithm of protein molecular weight vs. the relative mobility (R_f) of the protein (R_f = distance migrated by the protein/distance migrated by the dye front) or by using the point-to-point semilog interpolation method in Quantity One® or Image Lab™ software. Refer to bulletins 3133, 3144, and 10014472 for more information.

3.2 Mini-PROTEAN TGX Gels

Mini-PROTEAN TGX gels are Laemmli-like gels that have a proprietary modification that extends their shelf life to 12 months and enhances separation characteristics relative to conventional gel types. They are run using standard Laemmli sample buffer and Tris/glycine/SDS running buffer, and they generate protein migration patterns that are similar to those observed with standard Laemmli Tris-HCl gels. Mini-PROTEAN TGX gels are available in polyacrylamide single percentages and gradients. Use the protein migration charts and tables to select the gel type that optimizes resolution of your sample:

- Use single-percentage gels to separate bands of similar molecular weight. Optimum separation occurs in the lower half of the gel, so use a percentage in which the protein migrates to the lower half of the gel
- Use gradient gels to separate samples containing a broad range of molecular weights. Gradient gels allow resolution of both high- and low-molecular weight bands on the same gel. Larger pore sizes at the top of the gel permit resolution of larger molecules, smaller pore sizes toward the bottom of the gel restrict excessive separation of small molecules

Gel Percentage	Optimum Separation Range
7.5%	40–200 kD
10%	30–150 kD
12%	20–120 kD
4–15%	20–250 kD
4–20%	10–200 kD
Any kD™[1]	10–200 kD



Migration charts for protein standards on Mini-PROTEAN TGX gels.

[1] Any kD is a unique single-percentage formulation that provides a broad separation range and short running time.

3.3 SDS-PAGE Buffers

See Appendix B for buffer formulations. Do not adjust pH.

Running buffer (1x)

25 mM Tris, 192 mM glycine, 0.1% SDS

Dilute 100 ml 10x stock (catalog #161-0732) with 900 ml deionized water (diH_2O).

Sample buffer

62.5 mM Tris-HCl, pH 6.8, 2% SDS, 25% glycerol, 0.01% bromophenol blue, 5% β-mercaptoethanol or 350 mM DTT (added fresh)

Use Laemmli sample buffer (#161-0764) and add β-mercaptoethanol or DTT before use.

3.4 Sample Preparation

1. Determine the appropriate concentration of sample to load (depends on the load volume and the detection method used; see Chapter 9 for approximate stain sensitivities).
2. Dilute the sample with at least an equivalent volume of sample buffer with added reducing agent. For nonreducing conditions, omit the reducing agent.

   For example, combine:
   5 µl sample
   4.75 µl Laemmli (SDS-PAGE) sample buffer (catalog #161-0737)
   0.25 µl β-mercaptoethanol (catalog #161-0710)
   10 µl total volume

3. Heat the diluted sample at 90–95°C for 5 min or at 70°C for 10 min.

3.5 Running Conditions

Run conditions and times are approximate. Run times represent the time required for the dye front to reach the line at the bottom of the cassette. Conditions may vary depending on water and buffer conductivity, which vary from one lab setting to the next. Multiply current by the number of gels run.

Table 3.1. Standard running conditions for SDS-PAGE in the Mini-PROTEAN Tetra cell.

Gel	Optimum Range	Run Conditions	Run Time
7.5% 10% 12%	40–200 kD 30–150 kD 20–120 kD	200 V constant: Starting current (per gel): 37 mA Final current (per gel): 23 mA	38 min
4–15% 4–20% Any kD™	20–250 kD 10–200 kD 10–200 kD	200 V constant: Starting current (per gel): 50 mA Final current (per gel): 33 mA	28–30 min

Table 3.2. Alternative Running conditions for SDS-PAGE in the Mini-PROTEAN Tetra cell.

	100 V	200 V	300 V
Run time	85–95 min	31–39 min	15–23 min
Expected current (per gel)			
Initial	15–20 mA	25–50 mA	65–70 mA
Final	5–10 mA	20–31 mA	55–60 mA
Expected temperature	25°C	25–35°C	30–45°C
Outer buffer volume			
1–2 Gels	2-gel mark	2-gel mark	4-gel mark
3–4 Gels	4-gel mark	4-gel mark	4-gel mark[1]

[1] Requires the PowerPac HV or PowerPac Universal power supply.

Table 3.3. PowerPac™ power supply recommendations.

#Gels	100 V	200 V	300 V
1–2	Basic/HC/HV/Universal	Basic/HC/HV/Universal	Basic/HV/Universal
3–4	Basic/HC/HV/Universal	Basic/HC/HV/Universal	HV/Universal
4–8	HC/HV/Universal	HC/HV/Universal	Universal
9–10	HC/Universal	HC/Universal	Universal
11–12	HC/Universal	HC/Universal	Universal

4 Native PAGE

4.1 Introduction

In native PAGE, proteins are prepared in nonreducing, nondenaturing sample buffer, which maintains native structure and mass-to-charge ratios. Separation is also performed in the absence of SDS and reducing agents. Though native PAGE uses the same moving boundary described for SDS-PAGE (see Section 3.1), protein mobility depends on a number of factors other than molecular weight, including the shape and charge of the protein. Protein-protein interactions may be retained during native PAGE, so some proteins may separate as multisubunit complexes. Consequently, native PAGE is not suitable for molecular weight determination.

The nonreducing and nondenaturing environment of native PAGE allows protein separation with retention of biological activity. Because native structure is retained, native PAGE can enable separation of proteins with the same molecular weight.

4.2 Mini-PROTEAN TGX Gels

Mini-PROTEAN TGX™ gels are Laemmli-like gels that have a proprietary modification that extends their shelf life to 12 months and enhances separation characteristics relative to conventional gel types. They are run using standard native sample buffer and Tris/glycine running buffer, and they generate protein migration patterns that are similar to those observed with standard Laemmli Tris-HCl gels. Mini-PROTEAN TGX gels are available in a selection of polyacrylamide single percentages and gradients, and because they contain no sodium dodecyl sulfate (SDS), they can be used for either SDS- or native PAGE applications.

4.3 Native PAGE Buffers

See Appendix B for buffer formulations. Do not adjust pH.

Running buffer (1x) 25 mM Tris, 192 mM glycine
Dilute 100 ml 10x stock (catalog #161-0734) with 900 ml diH_2O.

Sample buffer 62.5 mM Tris-HCl, pH 6.8, 25% (v/v) glycerol, 0.01% (w/v) bromophenol blue (catalog # 161-0738)

4.4 Sample Preparation

In the absence of SDS, the net charge of a polypeptide is determined by its amino acid composition and the pH of the gel during electrophoresis, which is a function of the sample buffer, gel buffer, and running buffer. Only polypeptides with a net negative charge migrate into gels under native conditions. Most polypeptides have an acidic or slightly basic pI (~3–8). These proteins can be separated using the following standard protocol:

1. Determine the desired protein concentration and load volume of your sample based on the detection method used (see Chapter 9 for approximate stain sensitivities).
2. Dilute the sample with an equal volume of native sample buffer (**do not heat the samples**).

   For example, combine:
   5 µl sample
   5 µl native sample buffer (catalog #161-0738)
   10 µl total volume

Strongly basic proteins (pI >8.5) have a net positive charge and will not enter a Mini-PROTEAN TGX gel under native conditions using Tris/glycine buffer. To allow polypeptides with a net positive charge to migrate into a native gel, change the polarity of the electrodes by reversing the color-coded jacks when connecting to the power supply.

4.5 Running Conditions

Running conditions for native PAGE are similar to the standard running conditions used for SDS-PAGE (Section 3.4). If elevated temperature is a concern, run native PAGE at lower voltage; at lower voltages, runs require more time to complete.

Table 4.1. Standard running conditions for native PAGE with one (1) Mini-PROTEAN TGX gel in the Mini-PROTEAN Tetra cell. Run conditions and times are approximate and assume a constant voltage of 200 V. When running more than one gel, current will differ but temperature and run time should be close to those listed.

Gel	Current (mA) at 200 V Initial	Final	Temperature	Run Time
1 Gel (buffer to 2-gel mark)				
7.5% 10% 12%	35–37	17–20	28–30°C	38–40 min
4–15% 4–20% Any kD™	50–55	25–28	30–33°C	30–34 min

5 2-D Electrophoresis

5.1 Introduction

Mini-PROTEAN® precast gels are available for second-dimension PAGE in 2-D electrophoresis workflows. The IPG-well gels accommodate 7 cm IPG strips. Mini-PROTEAN® TGX Any kD™ gels are particularly well suited to 2-D electrophoresis applications.

The transition from first-to second-dimension gel electrophoresis involves:

- Equilibration of the resolved IPG strips in an SDS-containing, reducing buffer
- Placing the IPG strip on top of the second-dimension gel (agarose overlay)

5.2 Equilibration

Equilibration ensures that proteins in the IPG strips are coated with SDS and that cysteines are reduced and alkylated. Use the equilibration protocols (bulletin 411009) and buffers in the ReadyPrep™ 2-D starter kit (catalog #163-2105), or other protocols and buffers used for Tris-HCl gels.

5.3 Agarose Overlay

Place the equilibrated IPG strip into the IPG well of the gel and overlay it with molten agarose to ensure good contact between the strip and gel.

1. Prepare 0.5% low-melt agarose (catalog #161-3111), 0.003% bromophenol blue (catalog #161-0404) in 1x Tris/glycine/SDS running buffer (or use ReadyPrep overlay agarose, catalog #163-2111).
2. Following equilibration, place the IPG strip, gel side up, on the back plate of the gel, above the IPG well. The “+” and pH range on the IPG strip should be on the left.
3. Using forceps, push the strip into the IPG well, taking care to not trap air bubbles under the strip. Push on the backing of the strip, not on the gel.
4. Using a disposable pipet, apply overlay agarose into the IPG well. Fill the well to the top of the inner plate. Dispense rapidly, as overlay agarose solidifies quickly. To avoid bubbles, tilt the cassette slightly to allow bubbles to escape. Push gently on the plastic backings of the strip to free any trapped bubbles.

5.4 Second-Dimension Electrophoresis

Place the cassettes in to the Mini-PROTEAN® Tetra cell and start the run using the run conditions for SDS-PAGE. Use the migration of the bromophenol blue in the overlay agarose to monitor the progress of the run.

6 Peptide Analysis

6.1 Introduction

Mini-PROTEAN® Tris-Tricine peptide analysis gels are optimized for separating peptides and proteins with molecular weight <10,000. Peptide-SDS complexes move more slowly through these gels, allowing the faster SDS micelles, which normally interfere with peptide separations, to completely separate from peptides. This enables resolution of distinct peptide bands.

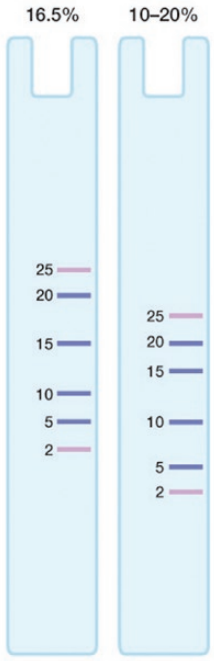
6.2 Mini-PROTEAN Tris-Tricine Gels

6.2.1 Gel Composition

Gel buffer	1.0 M Tris-HCl, pH 8.45
Crosslinker	2.6% C
Stacking gel	4% T, 2.6% C
Storage buffer	1.0 M Tris-HCl, pH 8.45, NaN_3
Shelf life	12 weeks at 2–8°C; expiration date is printed on each cassette

6.2.2 Gel Selection Guide

Gel Percentage	Optimum Separation Range
16.5%	1.5–30 kD
10–20%	1–40 kD



Migration charts for protein standards on Mini-PROTEAN Tris-Tricine gels.

6.3 Peptide Analysis Buffers

See Appendix B for buffer formulations. Do not adjust pH unless instructed to do so.

Running buffer (1x)	100 mM Tris, 100 mM Tricine, 0.1% SDS Dilute 100 ml 10x stock (catalog #161-0744) with 900 ml diH_2O
Sample buffer (catalog #161-0739)	200 mM Tris-HCl, pH 6.8, 2% SDS, 40% glycerol, 0.04% Coomassie Brilliant Blue G-250, 2% β-mercaptoethanol or 350 mM DTT (added fresh)

6.4 Sample Preparation

1. Determine the appropriate concentration of sample to load (depends on the load volume and the detection method used; see Chapter 9 for approximate stain sensitivities).
2. Dilute the sample with at least an equivalent volume of sample buffer (catalog #161-0739) and reducing agent (β-mercaptoethanol, for example). Heat the diluted sample at 90–95°C for 5 min, or at 70°C for 10 min.

   For example, combine:

   5 µl sample

   4.75 µl Tricine sample buffer (catalog #161-0739)

   0.25 µl β-mercaptoethanol (catalog #161-0710)

   10 µl total volume

6.5 Running Conditions

Table 6.1. Running conditions for one (1) Mini-PROTEAN Tricine gel in the Mini-PROTEAN Tetra cell. Run conditions and times are approximate and assume a constant voltage of 100 V. When running more than one gel, current will differ.

	16.5% Gels	10–20% Gels
Power conditions	100 V constant	100 V constant
Expected current (per gel)		
Initial	65 mA	35 mA
Final	65 mA	35 mA
Run time	100 min	100 min

7 Nondenaturing Nucleic Acid PAGE

7.1 Introduction

Mini-PROTEAN® TBE gels are used to separate small double-stranded DNA (dsDNA) fragments, particularly PCR products. DNA molecules have nearly uniform mass-to-charge ratios, allowing nondenaturing nucleic acid PAGE to separate dsDNA by mass using a continuous TBE buffer system.

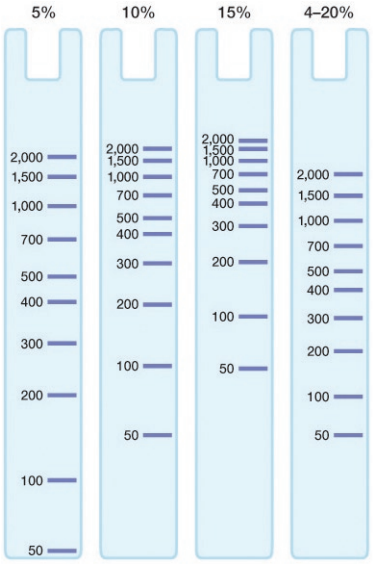
7.2 Mini-PROTEAN TBE Gels

7.2.1 Gel Composition

Gel buffer	89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3
Crosslinker	3.3% C
Stacking gel	4% T, 3.3% C
Storage buffer	89 mM Tris, 89 mM boric acid, 2 mM EDTA, NaN_3
Shelf life	12 weeks at 2–8°C; expiration date is printed on each cassette

7.2.2 Gel Selection Guide

Gel Percentage	Optimum Separation Range
5%	200–2,000 bp
10%	50–1,500 bp
15%	20–1,000 bp
4–20%	10–2,000 bp



Migration charts for protein standards on Mini-PROTEAN TBE gels.

7.3 Nondenaturing Nucleic Acid PAGE Buffers

See Appendix B for buffer formulations. Do not adjust pH unless directed to do so.

Running buffer (1x)	89 mM Tris, 89 mM boric acid, 2 mM EDTA Dilute 100 ml 10x stock (catalog #161-0733) with 900 ml diH_2O
Sample buffer (5x) (catalog #161-0767)	50 mM Tris-HCl, pH 8.0, 5 mM EDTA, 25% glycerol, 0.2% bromophenol blue, 0.2% xylene cyanole FF

7.4 Sample Preparation

Determine the DNA concentration of your sample based on the detection method used. (See Chapter 9 for approximate stain sensitivities.) Dilute 4 parts sample with 1 part sample buffer.

7.5 Running Conditions

Table 7.1. Running conditions for nondenaturing nucleic acid PAGE with one (1) Mini-PROTEAN TBE gel in the Mini-PROTEAN Tetra cell. Run conditions and times are approximate and assume a constant voltage of 100 V. When running more than one gel, current will differ.

	5% Gels	10% Gels	15% Gels	4–20% Gels
Power conditions	100 V constant	100 V constant	100 V constant	100 V constant
Expected current (per gel)				
Initial	15 mA	15 mA	15 mA	15 mA
Final	10 mA	10 mA	10 mA	10 mA
Run time	45–60 min	60–75 min	75–90 min	90–105 min

8 Denaturing Nucleic Acid PAGE

8.1 Introduction

Mini-PROTEAN® TBE-urea gels are used for separation of small RNA and single-stranded DNA (ssDNA) fragments. Applications include oligonucleotide analysis, RNase protection assays, and northern blotting.

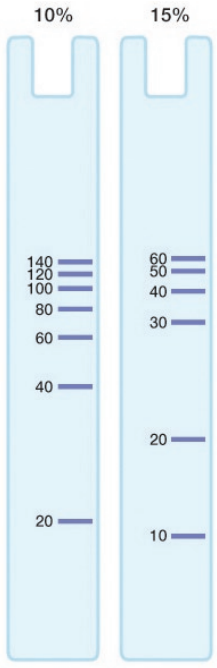
8.2 Mini-PROTEAN TBE-Urea Gels

8.2.1 Gel Composition

Gel buffer	89 mM Tris, 89 mM boric acid, 2 mM EDTA, 7 M urea, pH 8.3
Crosslinker	3.3% C
Stacking gel	4% T, 3.3% C
Storage buffer	89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3, NaN_3
Shelf life	8 weeks at 2–8°C; expiration date is printed on each cassette

8.2.2 Gel Selection Guide

Gel Percentage	Optimum Separation Range
10%	25–300 nt
15%	10–50 nt



Migration charts for protein standards on Mini-PROTEAN TBE-urea gels.

8.3 Denaturing Nucleic Acid PAGE Buffers

> See Appendix B for buffer formulations. Do not adjust pH unless directed to do so.

Running buffer (1x)	89 mM Tris, 89 mM boric acid, 2 mM EDTA Dilute 100 ml 10x stock (catalog #161-0733) with 900 ml diH_2O
Sample buffer (5x) (catalog #161-0768)	89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0, 12% Ficoll, 0.01% bromophenol blue, 0.02% xylene cyanole FF, 7 M urea

8.4 Sample Preparation

Determine the desired ssDNA or RNA concentration for your sample based on the detection method used. Dilute 4 parts sample with 1 part sample buffer.

8.5 Running Conditions

Table 8.1. Running conditions for denaturing nucleic acid PAGE with one (1) Mini-PROTEAN TBE-urea gel in the Mini-PROTEAN Tetra cell. Run conditions and times are approximate and assume a constant voltage of 200 V. When running more than one gel, current will differ.

	10% Gels	15% Gels
Power conditions	200 V constant	200 V constant
Expected current (per gel)		
Initial	15 mA	15 mA
Final	10 mA	10 mA
Run time	45–60 min	60–75 min

9 Detection

9.1 SDS-PAGE and Native PAGE Detection

Following electrophoresis, stain the gel to visualize the proteins. Refer to Table 9.1 for a comparison of total protein stains

Table 9.1. Total protein gel stains for use with Mini-PROTEAN gels.

Stain	Sensitivity (Lower Limit)	Optimum Protein Load (µg/Band)	Advantages	Disadvantages	Imaging	Manual
Mini-PROTEAN TGX Gels						
Coomassie R-250	36–47 ng	~0.5	Laboratory standard	Requires methanol destaining	Photography with white light or transmission densitometry	Consult literature
Bio-Safe™ Coomassie	8–28 ng	~0.5	Nonhazardous			4307051
Zinc stain[1]	6–12 ng	~0.2	High contrast, fast, reversible	Negative SDS-PAGE stain, must be photographed		4006082
Silver Stain Plus™ kit	0.6–1.2 ng	~0.01	Sensitive, robust, mass spectrometry compatible	Does not stain glycoproteins well		LIT442
Silver stain	0.6–1.2 ng	~0.01	Stains complex proteins (glyco- or lipoproteins)	Not mass spectrometry compatible		LIT34
Dodeca™ silver stain kit	0.5–1.2 ng	~0.1	Convenient staining for a large number of gels			4110150
Oriole™ fluorescent gel stain[1]	~2 ng	~0.1	High sensitivity, broad dynamic range, simple one-step protocol		Fluorescence visualization with UV trans-illumination	10017295
SYPRO Ruby protein gel stain	1–10 ng	~0.1	Broad dynamic range	Requires laser- or LED-based imaging instrument for maximum sensitivity	Fluorescence visualization with UV, LED, or laser scanning	4006173
Flamingo™ fluorescent gel stain	0.25–0.5 ng	~0.02	Broad dynamic range, mass spectrometry compatible			10003321

[1]Do not use zinc stain or Oriole fluorescent gel stain to stain native gels.

Stain	Sensitivity (Lower Limit)	Optimum Protein Load (µg/Band)	Advantages	Disadvantages	Imaging	Manual
Stain Free imaging	2–28 ng	~0.5	Rapid (<5 min), compatible with blotting and mass spectrometry, simple protocol with no additional reagents	Requires tryptophan residues in proteins for detection	Fluorescence using the Criterion Stain Free imaging system	10014472

9.2 Peptide Gel Staining

Peptides and small proteins are prone to diffusion and loss during staining. The following protocol includes a fixing step prior to staining to prevent sample loss and is suitable for detection of bands as low as 10–20 ng.

Fixative solution 40% methanol, 10% acetic acid

Stain solution 0.025% (w/v) Coomassie Blue G-250, 10% acetic acid

Destain solution 10% acetic acid

Place gels in fixative solution and equilibrate for 30 min. Stain gels with stain solution for 1 hr. Stain should be used only once; reuse may result in loss of sensitivity. Destain gels three times for 15 min or until the desired background is achieved. Some peptides may not be completely fixed and may diffuse out of the gels if fixing and staining times are greatly exceeded.

9.3 TBE Gel Staining

Use Table 9.2 as a guide to selecting an appropriate staining method.

Table 9.2. TBE gel detection methods.

Method	Sensitivity (Lower Limit)	Advantages	Disadvantages
Ethidium bromide	50 ng	Classic fluorescent DNA stain	Carcinogenic
Silver stain	1–2 ng	More sensitive than ethidium bromide	Requires multiple steps
SYBR® Green	0.02–2 ng	High sensitivity	Multiple steps, –20°C storage
SYBR® Safe	0.5 ng	Non-hazardous	Multiple steps

9.4 TBE-Urea Gel Staining

Use Table 9.3 as a guide to selecting an appropriate staining method.

Table 9.3. TBE-urea gel detection methods.

Method	Sensitivity (Lower Limit)	Advantages	Disadvantages
Ethidium bromide	10 ng	Classic fluorescent DNA stain	Carcinogenic
SYBR® Green	0.02–2 ng	High sensitivity	Requires multiple steps, –20°C storage
Silver stain	1–2 ng	More sensitive than ethidium bromide	Requires multiple steps

10 Blotting

10.1 Introduction

Western blotting is an electrophoretic technique used to move proteins from a gel onto a solid support, such as a nitrocellulose or PVDF membrane. The membrane can be used for immunological or biochemical analyses or demonstration of protein-protein or protein-ligand interactions.

Below are guidelines for western blotting of Mini-PROTEAN® precast gels onto nitrocellulose or PVDF membranes using either wet or semi-dry transfer techniques. Assess transfer efficiency using a total protein blot stain (see Table 9.1).

10.2 Transfer

10.2.1 Transfer Buffers

> See Appendix B for buffer formulations. Do not adjust pH unless directed to do so.

Towbin buffer (1x) 25 mM Tris, 192 mM glycine, 20% (v/v) methanol (pH 8.3)
Dilute 100 ml 10x stock (catalog #161-0734) with 400 ml diH_2O.
Add 200 ml methanol, then adjust volume to 1 L with diH_2O.

Add SDS to 0.1% to promote transfer of high molecular weight proteins.

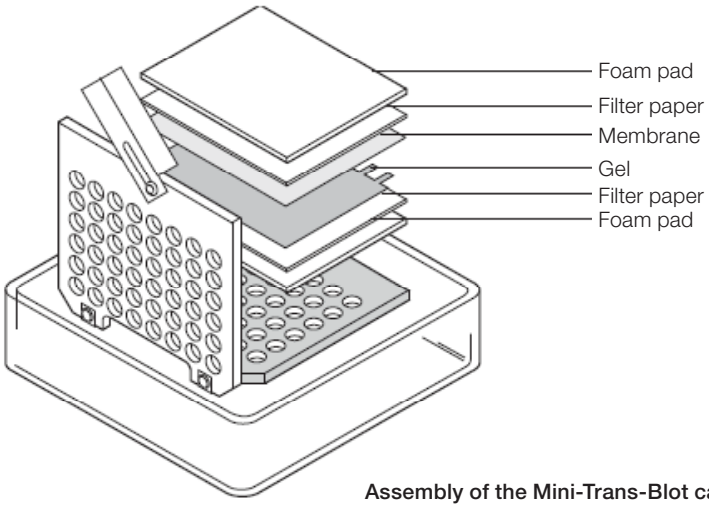
10.2.2 Wet Transfer Using the Mini Trans-Blot® Module

1. Equilibrate the gels in transfer buffer for 10–20 min prior to blot assembly.
2. Assemble the Mini Trans-Blot cassette. Place the gel closest to the black plate and the membrane closest to the red plate of the cassette. Use a roller to remove air trapped between the layers of the blot assembly.

> Wet PVDF membranes in methanol before soaking in transfer buffer.

3. Place the assembled cassette into the transfer module and tank. The red cassette plate should face the red side of the transfer module. Repeat steps 2 and 3 for a second blot, if needed.
4. Add the cooling unit and stirbar, and fill the tank with transfer buffer. Place the tank on a stir plate, and begin stirring to maintain even buffer temperature and ion concentration during the transfer.
5. Connect the Mini Trans-Blot cell to a suitable power supply and begin transfer.

For many proteins, excellent transfer efficiency is obtained in 30 min at a constant voltage of 100 V. For best results, optimize conditions for proteins of interest. Large proteins (>150 kD) may take 60 min, while smaller proteins (<30 kD) may transfer in 20 min. Refer to the Mini Trans-Blot Instruction Manual (bulletin 1703910) or the Protein Blotting Guide (bulletin 2895) for additional information.



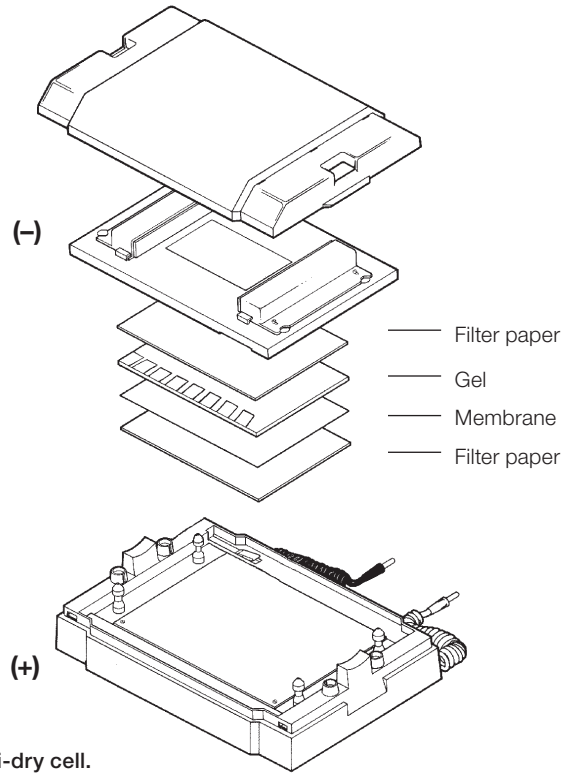
Assembly of the Mini-Trans-Blot cassette.

10.2.3 Semi-Dry Transfer Using the Trans-Blot® SD Cell

1. Equilibrate the gels in transfer buffer for 10–20 min.
2. Assemble the blot for transfer using the Trans-Blot SD semi-dry transfer system.
3. Connect the Trans-Blot SD cell to a PowerPac™ Basic power supply and begin transfer at 25 V.

For most proteins transferred from Mini-PROTEAN precast gels, optimum transfer efficiency is obtained in 30 min; smaller proteins (<30 kD) may transfer more quickly, while proteins >150 kD may show increased transfer efficiencies at up to 60 min. Run times longer than 60 min are NOT recommended for semi-dry transfers.

Refer to the Trans-Blot SD Instruction Manual (bulletin 1703940) or the Protein Blotting Guide (bulletin 2895) for additional information.



Assembly of the Trans-Blot SD semi-dry cell.

10.3 Total Protein Blot Stains

Total protein staining of a membrane provides an image of the complete protein pattern, which is required for the full characterization of specific antigens detected in complex protein mixtures. Gels shrink during staining, so comparison of an immunologically probed membrane to a stained gel is not practical. Instead, the exact location of a specifc antigen is determined by comparing two blotted membranes: one that has been probed with an antibody and the other stained for total protein.

Table 7.1. Total protein blot stains.

Method	Sensitivity	Protein Load (µg/Band)	Advantages	Disadvantages	Imaging
SYPRO Ruby protein blot stain	2–8 ng	~0.2	Compatible with mass spectrometry, Edman-based sequencing, and standard immunological procedures	Multistep protocol requires UV, LED, or laser imaging for maximum sensitivity	Fluorescence visualization with UV, LED epi-illumination or laser scanning
Colloidal gold stain	1 ng	~0.1	High sensitivity; single-step protocol	Incompatible with nylon membranes	Photography with epi-illumination or reflectance densitometry
Anionic dyes (amido black, Coomassie R-250, Ponceau S, Fast Green FCF)	100–1,000 ng	~5.0	Inexpensive, rapid	Low sensitivity	

10.4 Immunodetection

After transfer, blots are ready for downstream processing. Though all protein and antibody combinations are different and may require optimization, a general protocol for immunodetection of a large number of protein and antibody combinations is listed below. See Appendix B for buffer formulations.

1. Immediately after transfer, place the membrane into Tris-buffered saline with Tween 20 (TTBS) containing blocking agent (for example, 3% BSA, 5% nonfat dry milk, 1% casein, or 1% gelatin) and incubate either for 1 hr at room temperature or overnight at 4°C.
2. Dilute the primary antibody in blocking solution (dilution is specified by the manufacturer). Incubate at room temperature with agitation for 1 hr.
3. Wash the blot with TTBS as directed (for example, five times, 5 min each at room temperature with agitation).
4. Dilute the secondary antibody into TTBS as specified by the manufacturer. Incubate the blot in the secondary solution at room temperature with agitation for 1 hr.
5. Wash the blot with TTBS five times, 5 min each at room temperature with agitation.
6. Follow the directions for the detection kit used to develop the blot. For the Immun-Star™ WesternC™ chemiluminescence kit (catalog #170-5070), mix 3 ml luminol/enhancer with 3 ml peroxide solution to make a 1x working solution for a 7 x 8.5 cm membrane. Incubate the membrane in the solution for 3–5 min. Prior to imaging, drain the excess substrate and place the membrane in a protective sleeve (such as plastic wrap) to prevent drying.

11 Troubleshooting

Table 11.1. Troubleshooting electrophoresis and detection with Mini-PROTEAN® gels. For more troubleshooting tips, refer to the Mini-PROTEAN Tetra, Mini Trans-Blot®, and Trans-Blot® SD instruction manuals, or contact Technical Support.

Problem	Cause	Solution
Current is zero or less than expected, and samples do not migrate into gel	Tape at bottom of cassette not removed	Remove tape
	Insufficient buffer in inner buffer chamber	Fill buffer chamber with running buffer
	Insufficient buffer in outer buffer chamber	Fill inner and outer chambers to ensure wells of the gels are completely covered
	Incorrect cassette orientation	Ensure shorter plate is facing gasket
	Electrical disconnection	Check electrodes and connections
Gels run faster than expected	Running buffer too concentrated or incorrect	Check buffer composition
	Gel temperature too high	Do not exceed recommended running conditions
Gels run more slowly than expected	Companion running module left in tank when running only 1–2 gels	Remove companion running module
Buffer leaking from inner chamber	Incomplete gasket seal	Wet gasket with running buffer before use
	Improper assembly of the gel into the electrode/companion module	Top edge of short plate should fit under notch at top of gasket
		Top of short plate should touch green gasket
Bands “smile” across gel: band pattern curves upward at both sides of gel	Excessive heating of gel	Check buffer composition
		Do not exceed recommended running conditions
	Insufficient buffer	Fill inner and outer chambers to ensure wells of gels are completely covered
Bands “smile” or “frown” within gel lanes	Protein load too high	Load less protein
	Sample or buffer preparation issues	Minimize salts, detergents, and solvents in sample preparation and sample loading buffers
	Incorrect running conditions	Set correct voltage

Problem	Cause	Solution
Bands are skewed or distorted; lateral band spreading	Too much salt in samples	Remove salt from samples (dialysis, precipitation, or other method)
	Insufficient or wrong sample buffer	Check buffer composition and dilution
	Sample precipitation	Selectively remove predominant proteins
		Dilute sample in sample buffer
	Insoluble materials (for example, cell membranes) in samples	Centrifuge samples to remove particulates prior to sample loading
Artifactual bands at 60–70 kD	Skin keratin contamination	Clean all dishware; wear gloves while handling and loading gels
		Filter all solutions (0.2–0.45 µm filter)
Poor resolution or fuzzy bands	Sample volume too high	If possible, load a more concentrated sample in a lower sample buffer volume
	Diffuse sample loading zone	Load sample with a syringe or gel loading pipet tip
	Sample diffusion during staining with Coomassie	Fix gel with 40% methanol, 10% acetic acid for 80 min prior to staining
	Incompatible sample components	Minimize salts, detergents, and solvents in sample preparation and loading buffers
	Expired gel	Use gels before expiration date on cassette

A Quick Start Guides

This section contains abbreviated protocls (quick start guides) for the following electrophoretic techniques. Directions are for use of Mini-PROTEAN® precast gels and the Mini-PROTEAN® Tetra cell.

- SDS-PAGE using Mini-PROTEAN® TGX™ precast gels
- Native PAGE using Mini-PROTEAN TGX precast gels
- Peptide analysis using Mini-PROTEAN Tris-tricine gels
- Nondenaturing PAGE of nucleic acids using Mini-PROTEAN TBE gels
- Denaturing PAGE of nucleic acids using Mini-PROTEAN TBE-urea gels

SDS-PAGE (Mini-PROTEAN TGX Gels)

Prepare Buffers

Running buffer (1x) Dilute 100 ml 10x stock (catalog #161-0732) with 900 ml diH_2O.

Sample buffer Use Laemmli sample buffer (catalog #161-0737)

▼

Prepare Gels and Assemble Electrophoresis Cell

Remove the comb and tape from the gels and assemble the electrophoresis cell.

Fill the inner and outer buffer chambers with running buffer.

▼

Prepare and Load Samples

Component	Reducing	Nonreducing
Sample	5 µl	5 µl
Laemmli sample buffer (catalog #161-0737)	4.75 µl	5 µl
β-Mercaptoethanol	0.25 µl	—
Total volume	10 µl	10 µl

Heat samples at 90–100°C for 5 min (or at 70°C for 10 min).

Load the appropriate amount of sample on the gel.

▼

Perform Electrophoresis

Connect the electrophoresis cell to the power supply and perform electrophoresis according to the conditions in the table.

Table A.1. Running conditions for SDS-PAGE in the Mini-PROTEAN Tetra cell. Standard conditions are constant 200 V.

	100 V	200 V	300 V
Run time	85–95 min	31–39 min	15–23 min
Expected current (per gel)			
Initial	15–20 mA	25–50 mA	65–70 mA
Final	5–10 mA	20–31 mA	55–60 mA
Expected temperature	25°C	25–35°C	30–45°C
Outer buffer volume			
1–2 Gels	2-gel mark	2-gel mark	4-gel mark
3–4 Gels	4-gel mark	4-gel mark	4-gel mark[1]

[1] Requires the PowerPac™ HV or PowerPac Universal power supply.

Native PAGE (Mini-PROTEAN TGX Gels)

Prepare Buffers

Running buffer (1x) Dilute 100 ml 10x stock (catalog #161-0734) with 900 ml diH_2O.

Sample buffer Use native sample buffer (catalog #161-0738)

▼

Prepare Gels and Assemble Electrophoresis Cell

Remove the comb and tape from the gels and assemble the electrophoresis cell.

Fill the inner and outer buffer chambers with running buffer.

▼

Prepare and Load Samples

Component	Volume
Sample	5 µl
Native sample buffer (catalog #161-0738)	5 µl
Total volume	10 µl

Load the appropriate amount of sample on the gel.

▼

Perform Electrophoresis

Connect the electrophoresis cell to the power supply and perform electrophoresis according to the conditions in the table.

Table A.2. Standard running conditions for native PAGE with one (1) Mini-PROTEAN TGX gel in the Mini-PROTEAN Tetra cell. Run conditions and times are approximate and assume a constant voltage of 200 V. When running more than one gel, current will differ but temperature and run time should be close to those listed.

Gel	Current (mA) at 200 V Initial	Final	Temperature	Run Time
1 Gel (buffer to 2-gel mark)				
7.5% 10% 12%	35–37	17–20	28–30°C	38–40 min
4–15% 4–20% Any kD™	50–55	25–28	30–33°C	30–34 min

Peptide Analysis (Mini-PROTEAN Tris-Tricine Gels)

Prepare Buffers

Running buffer (1x) Dilute 100 ml 10x stock (catalog #161-0744) with 900 ml diH_2O.

Sample buffer (catalog #161-0739) 200 mM Tris-HCl, pH 6.8, 2% SDS, 40% glycerol, 0.04% Coomassie Brilliant Blue G-250, 2% ß-mercaptoethanol or 350 mM DTT (added fresh)

▼

Prepare Gels and Assemble Electrophoresis Cell

Remove the comb and tape from the gels and assemble the electrophoresis cell.

Fill the inner and outer buffer chambers with running buffer.

▼

Prepare and Load Samples

Component	Reducing	Nonreducing
Sample	5 µl	5 µl
Sample buffer (catalog #161-0739)	4.75 µl	5 µl
β-Mercaptoethanol	0.25 µl	—
Total volume	10 µl	10 µl

Heat samples at 90–100°C for 5 min or at 70°C for 10 min.

Load the appropriate amount of sample on the gel.

▼

Perform Electrophoresis

Connect the electrophoresis cell to the power supply and perform electrophoresis according to the conditions in the table.

Table A.3. Running conditions for one (1) Mini-PROTEAN Tricine gel in the Mini-PROTEAN Tetra cell. Run conditions and times are approximate and assume a constant voltage of 100 V. When running more than one gel, current will differ.

	16.5% Gels	10–20% Gels
Power conditions	100 V constant	100 V constant
Expected current (per gel)		
Initial	65 mA	35 mA
Final	65 mA	35 mA
Run time	100 min	100 min

Nondenaturing Nucleic Acid PAGE (Mini-PROTEAN TBE Gels)

Prepare Buffers

Running buffer (1x) Dilute 100 ml 10x stock (catalog #161-0733) with 900 ml diH_2O.

Sample buffer (5x) (catalog #161-0767) 50 mM Tris-HCl, pH 8.0, 5mM EDTA, 25% glycerol, 0.2% bromophenol blue, 0.2% xylene cyanole FF

▼

Prepare Gels and Assemble Electrophoresis Cell

Remove the comb and tape from the gels and assemble the electrophoresis cell.

Fill the inner and outer buffer chambers with running buffer.

▼

Prepare and Load Samples

Component	Amount
Sample	8 µl
Sample buffer (catalog #161-0767)	2 µl
Total volume	10 µl

Load the appropriate amount of sample on the gel.

▼

Perform Electrophoresis

Connect the electrophoresis cell to the power supply and perform electrophoresis according to the conditions in the table.

Table A.4. Running conditions for nondenaturing nucleic acid PAGE with one (1) Mini-PROTEAN TBE gel in the Mini-PROTEAN Tetra cell. Run conditions and times are approximate and assume a constant voltage of 100 V. When running more than one gel, current will differ.

		5% Gels	10% Gels	15% Gels	4–20% Gels
Power conditions		100 V constant	100 V constant	100 V constant	100 V constant
Expected current (per gel)					
	Initial	15 mA	15 mA	15 mA	15 mA
	Final	10 mA	10 mA	10 mA	10 mA
Run time	45–60 min	60–75 min	75–90 min	90–105 min	

Denaturing Nucleic Acid PAGE (Mini-PROTEAN TBE-Urea Gels)

Prepare Buffers

Running buffer (1x) Dilute 100 ml 10x stock (catalog #161-0733) with 900 ml diH_2O.

Sample buffer (5x) (catalog #161-0768) 89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0, 12% Ficoll, 0.01% bromophenol blue, 0.02% xylene cyanole FF, 7 M urea

▼

Prepare Gels and Assemble Electrophoresis Cell

Remove the comb and tape from the gels and assemble the electrophoresis cell.

Fill the inner and outer buffer chambers with running buffer.

▼

Prepare and Load Samples

Component	Amount
Sample	8 µl
Sample buffer (catalog #161-0768)	2 µl
Total volume	10 µl

Load the appropriate amount of sample on the gel.

▼

Perform Electrophoresis

Connect the electrophoresis cell to the power supply and perform electrophoresis according to the conditions in the table.

Table A.5. Running conditions for denaturing nucleic acid PAGE with one (1) Mini-PROTEAN TBE-urea gel in the Mini-PROTEAN Tetra cell. Run conditions and times are approximate and assume a constant voltage of 200 V. When running more than one gel, current will differ.

	5% Gels	10% Gels	15% Gels
Power conditions	200 V constant	200 V constant	200 V constant
Expected current (per gel)			
Initial	15 mA	15 mA	15 mA
Final	10 mA	10 mA	10 mA
Run time	30–45 min	45–60 min	60–75 min

B Buffers

Running Buffers

10x SDS-PAGE (1 L)
(catalog #161-0732)

250 mM Tris, 1.92 M glycine, 1% SDS, pH 8.3

Tris base	30.3 g
Glycine	144.1 g
SDS	10 g
diH_2O	to 1 L

Do not adjust the pH (~pH 8.3)

10x Native PAGE (1 L)
(catalog #161-0734)

250 mM Tris, 1.92 M glycine, pH 8.3

Tris base	30.3 g
Glycine	144.1 g
diH_2O	to 1 L

Do not adjust the pH (~pH 8.3)

10x Tris-Tricine (1 L)
(catalog #161-0744)

1 M Tris, 1 M Tricine, 1% SDS, pH 8.3

Tris base	121.1 g
Tricine	179.2 g
SDS	10 g
diH_2O	to 1 L

Do not adjust the pH (~pH 8.3)

10x TBE (1 L)
(catalog #161-0741)

890 mM Tris, 890 mM boric acid, 20 mM EDTA

Tris base	107.8 g
Boric acid	55.0 g
EDTA	5.8 g
diH_2O	to 1 L

Do not adjust the pH (~pH 8.3)

Sample Buffers

2x SDS-PAGE (Laemmli, 30 ml)
(catalog #161-0737)

62.5 mM Tris-HCl, pH 6.8, 2% SDS, 25% glycerol, 0.01% bromophenol blue, 5% ß-mercaptoethanol (added fresh)

0.5 M Tris-HCl, pH 6.8	3.75 ml
50% Glycerol	15.0 ml
1.0% Bromophenol blue	0.3 ml
10% SDS	6.0 ml
diH_2O	to 30 ml

Add β-mercaptoethanol (50 µl to 950 µl sample buffer) before use.

2x Native PAGE (30 ml)
(catalog #161-0738)

62.5 mM Tris-HCl, pH 6.8, 40% glycerol, 0.01% bromophenol blue

0.5 M Tris-HCl, pH 6.8	3.75 ml
50% Glycerol	24 ml
1.0% Bromophenol blue	0.3 ml
diH_2O	to 30 ml

2x Tricine (30 ml)
(catalog #161-0739)

200 mM Tris-HCl, pH 6.8, 2% SDS, 40% glycerol, 0.04% Coomassie Brilliant Blue G-250, 2% ß-mercaptoethanol (added fresh)

0.5 M Tris-HCl, pH 6.8	12.0 ml
50% Glycerol	24.0 ml
10% SDS	6.0 ml
Coomassie Blue G-250	12.0 mg
diH_2O	to 30 ml

Add β-mercaptoethanol (20 µl to 980 µl sample buffer) before use.

5x Nucleic acid (10 ml)
(catalog #161-0767)

50 mM Tris-HCl, pH 8.0, 5 mM EDTA, 25% glycerol, 0.2% bromophenol blue, 0.2% xylene cyanole FF

Tris base	78.8 mg
50% Glycerol	5 ml
EDTA	14.6 mg
1.0% Bromophenol blue	2.0 ml
Xylene cyanole FF	20.0 mg
diH_2O	to 10 ml

TBE-urea (30 ml) — **89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0, 12% Ficoll, 0.01% bromophenol blue, 0.02% xylene cyanole, 7 M urea**
(catalog #161-0768)
Store at 4°C

Tris base	0.32 g
Boric acid	0.165 g
EDTA	17.5 mg
Ficoll	3.6 g
Bromophenol blue	3 mg
Xylene cyanole FF	6 mg
Urea	12.6 g
diH_2O	to 30 ml

Buffer Components

0.5 M Tris-HCl, pH 6.8 (1 L)
(catalog #161-0799)
Store at 4°C

Tris base	60.6 g
diH_2O	~900 ml
Adjust to pH 6.8 with HCl	
diH_2O	to 1 L

10% SDS (250 ml)
(catalog #161-0416)

SDS	25.0 g
diH_2O	to 250 ml

1.0% Bromophenol blue (10 ml)
(10 g powder, catalog #161-0404)

Bromophenol blue	100 mg
diH_2O	to 10 ml

Blotting Buffers

Towbin buffer (1 L) — **25 mM Tris, 192 mM glycine, 20% methanol**

Dissolve:

Tris base	14.4 g
Glycine	3.03 g
diH_2O	500 ml

Then add:

Methanol	200 ml
diH_2O	to 1 L

Alternatively, use prepared stock solutions:

10x Tris/glycine (catalog #170-0734)	100 ml
Add 200 ml methanol and diH_2O to 1 L as above	

Tris-buffered saline with Tween (TTBS, 1 L) — **20 mM Tris, 500 mM NaCl, 0.05% Tween 20**

Tris base	2.4 g
NaCl	29.2 g
10% Tween 20	5.0 ml
diH_2O	to 1 L

Alternatively, use prepared stock solutions:

10x TBS (catalog #170-6435)	100 ml
10% Tween 20 (catalog #166-2404)	5 ml
diH_2O	895 ml

C Related Literature

Bulletin #	Title
10007296	Mini-PROTEAN® Tetra Cell Instruction Manual
4006191	Mini-PROTEAN 3 Dodeca™ Cell Instruction Manual
1703930	Mini Trans-Blot® Instruction Manual
4006066	Trans-Blot® SD Semi-Dry Transfer Cell Quick Reference Guide
1703940	Trans-Blot SD Semi-Dry Transfer Cell Instruction Manual
5871	Mini-PROTEAN® TGX™ Precast Gels Product Information Sheet
5535	Mini-PROTEAN Tetra Cell Brochure
2895	Protein Blotting Guide
3133	Molecular Weight Determination by SDS-PAGE
3144	Using Precision Plus Protein™ Standards to Determine Molecular Weight
1939	Blotting Membrane Brochure
2032	Western Blotting Detection Reagent Brochure
2317	Ready-to-Run Buffers and Solutions Brochure
2414	The Little Book of Standards

D Ordering Information

Mini-PROTEAN® TGX™ Precast Gels

	10 Gels per Box			
	10-Well[1]		15-Well	IPG/Prep
	(30 µl/well)	(50 µl/well)	(15 µl/well)	7 cm IPG strip (450 µl)
7.5%	456-1023	456-1024	456-1026	456-1021
10%	456-1033	456-1034	456-1036	456-1031
12%	456-1043	456-1044	456-1046	456-1041
4–15%	456-1083	456-1084	456-1086	456-1081
4–20%	456-1093	456-1094	456-1096	456-1091
Any kD™	456-9033	456-9034	456-9036	456-9031

[1]Gels can also be ordered with 2 gels per box by adding an "S" to the end of any of the above catalog numbers.

Mini-PROTEAN Tris-Tricine Precast Gels

	2 Gels per Box		
	10-Well		15-Well
	(30 µl/well)	(50 µl/well)	(15 µl/well)
16.5%	456-3063	456-3064	456-3066
10–20%	456-3113	456-3114	456-3116

Mini-PROTEAN TBE-Urea Precast Gels

	2 Gels per Box	
	10-Well	15-Well
	(30 µl/well)	(15 µl/well)
10%	456-6033	456-6036
15%	456-6053	456-6056

Mini-PROTEAN TBE Precast Gels

	2 Gels per Box				2 Gels per Box		
	10-Well		15-Well		10-Well		15-Well
	(30 µl/well)	(50 µl/well)	(15 µl/well)		(30 µl/well)	(50 µl/well	(15 µl/well)
5%	456-5013	456-5014	456-5016	15%	456-5053	456-5054	456-5056
10%	456-5033	456-5034	456-5036	4–20%	456-5093	456-5094	456-5096

Catalog #	Description
Protein Standards	
161-0363	Precision Plus Protein™ Unstained Standards (10–250 kD), 1 ml, 100 applications
161-0373	Precision Plus Protein All Blue Standards (10–250 kD), 500 µl, 50 applications
161-0374	Precision Plus Protein Dual Color Standards (10–250 kD), 500 µl, 50 applications
161-0375	Precision Plus Protein™ Kaleidoscope™ Standards (10–250 kD), 500 µl, 50 applications
161-0376	Precision Plus Protein™ WesternC™ Standards (10–250 kD), 250 µl, 50 applications
161-0377	Precision Plus Protein Dual Xtra Standards (2–250 kD), 500 µl, 50 applications
161-0385	Precision Plus Protein WesternC Pack (10–250 kD), 50 applications each of standard and StrepTactin-HRP
161-0317	SDS-PAGE Standards, broad range, 200 µl
Equipment	
165-8004	Mini-PROTEAN® Tetra Cell for Ready Gel™ Precast Plates (4-gel system)
165-8005	Mini-PROTEAN Tetra Cell for Ready Gel Precast plates (2-gel system)
165-4100	Mini-PROTEAN® 3 Dodeca™ Cell
170-3930	Mini Trans-Blot® Electrophoretic Transfer Cell
170-3940	Trans-Blot® SD Semi-Dry Electrophoretic Transfer Cell
164-5050	PowerPac™ Basic Power Supply
164-5052	PowerPac HC High Current Power Supply
Premixed Running Buffers	
161-0732	10x Tris/Glycine/SDS, 1 L
161-0772	10x Tris/Glycine/SDS, 5 L
161-0734	10x Tris/Glycine, 1 L
161-0744	10x Tris/Tricine/SDS, 1 L
161-0733	10x Tris/Boric Acid/EDTA, 1 L
161-0770	10x Tris/Boric Acid/EDTA, 5 L
Premixed Sample Buffers	
161-0737	Laemmli Sample Buffer, 30 ml[1]
161-0738	Native Sample Buffer, 30 ml
161-0739	Tricine Sample Buffer, 30 ml
161-0767	Nucleic Acid Sample Buffer, 5x, 10 ml
161-0768	TBE-Urea Sample Buffer, 30 ml

[1] May require addition of 2-mercaptoethanol or DTT

Catalog #	Description
Component Reagents	
161-0719	Tris, 1 kg
161-0718	Glycine, 1 kg
161-0301	SDS, 100 g
161-0416	SDS Solution, 10% (w/v), 250 ml
166-2404	10% Tween 20, 5 ml
170-6404	Blotting-Grade Blocker, 300 g
161-0710	2-Mercaptoethanol, 25 ml
161-0611	Dithiothreitol, 5 g
161-0404	Bromophenol Blue, 10 g
Blotting Membranes	
162-0212	0.2 µm Nitrocellulose/Filter Paper Sandwich, 7 x 8.5 cm, 20 pack
162-0213	0.2 µm Nitrocellulose/Filter Paper Sandwich, 7 x 8.5 cm, 50 pack
162-0214	0.45 µm Nitrocellulose/Filter Paper Sandwich, 7 x 8.5 cm, 20 pack
162-0215	0.45 µm Nitrocellulose/Filter Paper Sandwich, 7 x 8.5 cm, 50 pack
162-0216	Sequi-Blot™ PVDF/Filter Paper Sandwich, 7 x 8.5 cm, 20 pack
162-0217	Sequi-Blot PVDF/Filter Paper Sandwich, 7 x 8.5 cm, 50 pack
Total Protein Gel and Blot Stains	
161-0786	Bio-Safe™ Coomassie Stain, 1 L
161-0400	Coomassie Brilliant Blue R-250, 10 g
161-0436	Coomassie Brilliant Blue R-250 Stain Solution, 1 L
161-0438	Coomassie Brilliant Blue R-250 Destain Solution, 1 L
161-0443	Silver Stain Kit
161-0449	Silver Stain Plus™ Kit
170-6527	Colloidal Gold Total Protein Stain, 500 ml
161-0440	Zinc Stain and Destain Kit
170-3127	SYPRO Ruby Protein Blot Stain, 200 ml
161-0491	Flamingo™ Fluorescent Gel Stain (10x), 100 ml
161-0496	Oriole™ Fluorescent Protein Gel Stain (1x), 1 L

Catalog #	Description
Immunoblot Detection Reagents	
170-5070	Immun-Star™ WesternC™ Chemiluminescent Kit, 100 ml
170-6431	HRP Conjugate Substrate Kit, 4CN
170-6535	HRP Color Development Reagent, DAB, 5 g
170-8238	Amplified Opti-4CN™ Substrate Kit
170-8235	Opti-4CN Substrate Kit
170-6432	AP Conjugate Substrate Kit
170-5012	Immun-Star™ AP Substrate Pack

For additional product sizes, please refer to the Bio-Rad catalog or website.

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 31 3689 6600* ***Canada*** *905 364 3435* ***China*** *86 20 8732 2339* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 31 884 0* ***Greece*** *30 210 777 4396* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 459 6100* ***India*** *91 124 4029300* ***Israel*** *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666* ***New Zealand*** *0508 805 500* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55* ***Taiwan*** *886 2 2578 7189* ***United Kingdom*** *020 8328 2000*

Bulletin 1658100 Rev C US/EG *1110 Sig 1109*