Criterion™ Precast Gels

Instruction Manual and Application Guide

BIO-RAD

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Criterion™ TGX Stain-Free™ and Criterion Stain Free™ precast gels are covered by U.S. Patent No. 7,569,130.

Purchase of Criterion™ XT Bis-Tris gels, XT MOPS running buffer, XT MES running buffer, XT MOPS buffer kit, and XT MES buffer kit is accompanied by a limited license under U.S. patents 6,143,154; 6,096,182; 6,059,948; 5,578,180; 5,922,185; 6,162,338; and 6,783,651 and corresponding foreign patents.

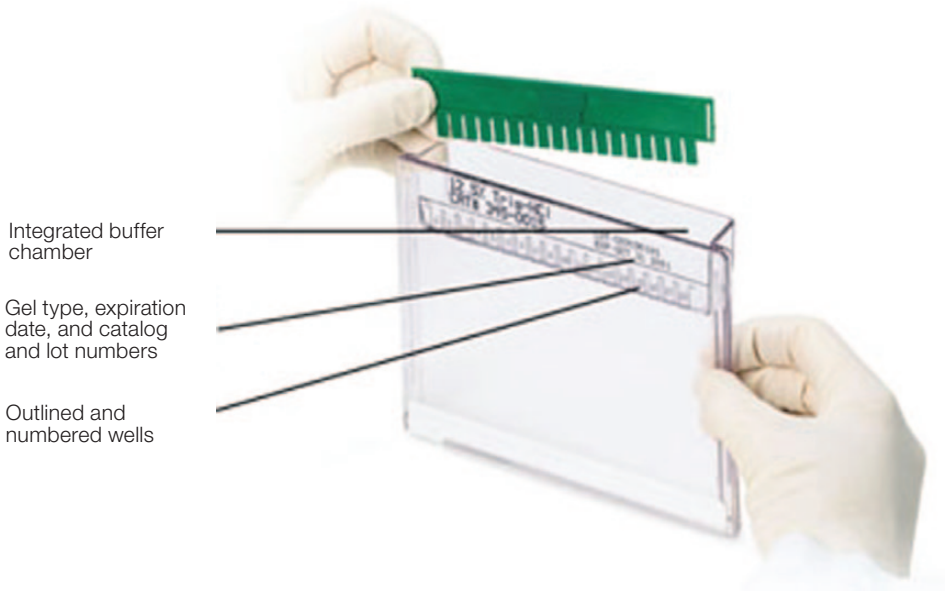
Contents

1 Criterion™ Precast Gels

1.1 Introduction

Criterion precast gels are an effective system for performing polyacrylamide gel electrophoresis (PAGE). These 13.3 x 8.7 cm gels are wider and longer than traditional mini format gels, and their innovative, easy-to-use design produces excellent resolution while accommodating more samples per gel. Designed for use with the Criterion family of vertical electrophoresis cells, which includes the Criterion (2-gel capacity) and Criterion™ Dodeca™ (12-gel capacity) cells, Criterion precast gels allow separation of more samples than mini format gels and provide significant cost and time savings. Some of the unique features provided are:

- Integrated buffer chamber that eliminates buffer leaks
- Capacity for up to 26 samples per gel
- Compatibility with multichannel pipets (12+2 and 26-well)
- Outlined and numbered wells that simplify sample loading and identification
- Patented[1] J-foot design that eliminates post-run gel processing steps and improves gel drying and blotting results
- Criterion™ TGX Stain-Free™ formulations for rapid gel imaging without staining



[1] U.S. patent 6,093,301.

1.2 Gel Formulations

Criterion precast gels are available in a range of formulations for virtually every electrophoresis application (Table 1.1). All Criterion gels are composed of polyacrylamide with a bisacrylamide crosslinker, and they are available in a selection of single percentages and gradients.

Table 1.1. Criterion precast gel formulations.

Application	Gel Formulation	Sample Buffer	Running Buffer
SDS-PAGE	Criterion Tris-HCl Criterion Stain Free Criterion TGX™ Criterion TGX Stain-Free	Laemmli	Tris/glycine/SDS
	Criterion™ XT Bis-Tris	XT	XT MOPS or XT MES
	Criterion XT Tris-acetate	XT	XT Tricine
Native PAGE	Criterion Tris-HCl Criterion Stain Free Criterion TGX Criterion TGX Stain-Free Criterion XT Tris-acetate	Native	Tris/glycine
Peptide analysis	Criterion Tris-Tricine	Tricine	Tris/Tricine/SDS
Isoelectric focusing (IEF)	Criterion IEF	IEF	Anode and cathode buffers
Protease detection	Criterion zymogram	Zymogram	Tris/glycine/SDS
dsDNA separation	Criterion TBE	Nucleic acid	Tris/boric acid/EDTA (TBE)
ssDNA and RNA separation	Criterion TBE-urea	TBE-urea	TBE

1.3 Comb Configurations

Comb Type	Well Volume
12+2 well[1]	45 µl with two 15 µl reference wells
18-well	30 µl
26-well[1]	15 µl
Prep+2 well	800 µl with two 15 µl reference wells
IPG+1 well	11 cm ReadyStrip™ IPG strip (450 µl) with one 15 µl reference well

1.4 Specifications

Gel material	Polyacrylamide
Gel dimensions (W x L)	13.3 x 8.7 cm
Gel thickness	1.0 mm
Resolving gel height	6.5 cm
Cassette dimensions (W x L)	15.0 x 10.6 cm
Cassette material	Styrene copolymer
Comb material	Polycarbonate
Running buffer	460 ml (per gel)

[1] Multichannel pipet compatible.

1.5 Storage Conditions

Table 1.2. Storage conditions for Criterion 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
Ambient	Criterion XT Bis-Tris	12 months
2–8°C	Criterion TGX	12 months
	Criterion TGX Stain-Free	12 months
	Criterion Tris-HCl	12 weeks
	Criterion Stain-Free	12 weeks
	Criterion XT Tris-acetate	8 months
	Criterion Tris-Tricine	12 weeks
	Criterion IEF	24 weeks
	Criterion zymogram	8 weeks
	Criterion TBE	12 weeks
	Criterion TBE-urea	8 weeks

1.6 Important Notes

Use each Criterion precast gel as soon as possible after removing it from the storage pouch.

Improper storage of Criterion precast gels can produce numerous artifacts.

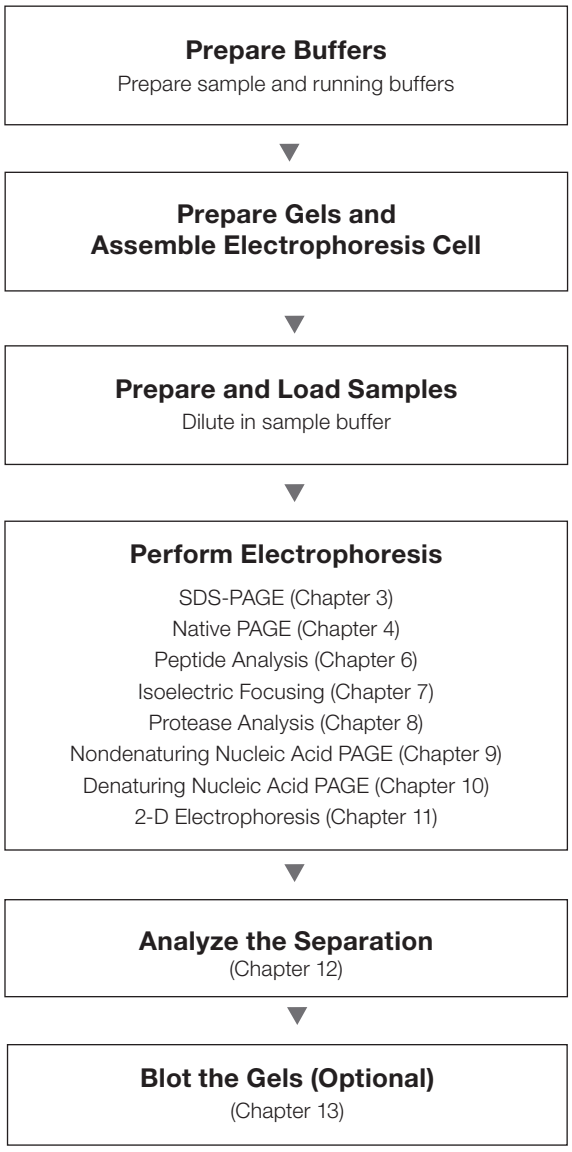
- Store gels flat
- Avoid prolonged storage at temperatures above those recommended
- Do not freeze gels
- If you suspect your gels have been stored improperly, discard them

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.

Use unstained standards with Criterion TGX Stain-Free and Criterion Stain Free gels, as some prestained standards are not detected by the Gel Doc™ EZ imager. To monitor electrophoresis, use 10 µl of a 1:1 mixture of Precision Plus Protein™ unstained and prestained standards.

2 Setup and Basic Operation

2.1 Workflow Overview



2.2 Required Materials

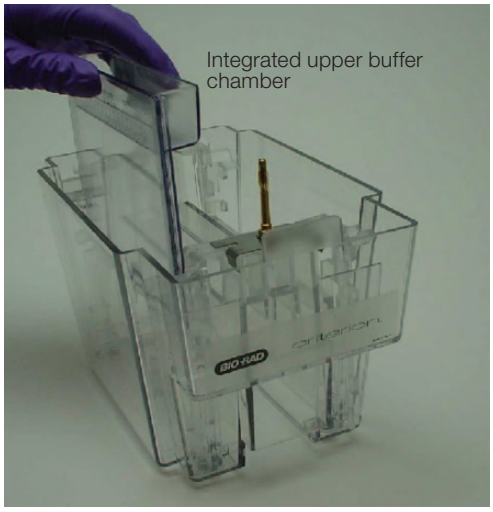
- Criterion™ precast gels
- Criterion or Criterion™ Dodeca™cell
- PowerPac™ Basic or PowerPac HC power supply (or equivalent)
- Sample buffer
- Running buffer (460 ml per gel)

2.3 Setting Up and Running Criterion Gels in the Criterion Cell

1. Each Criterion gel is packaged in a plastic storage tray. Remove the cover of the tray by lifting the corner tab and pulling it diagonally across the package. Remove the gel from the package.
2. Remove the comb and rinse the wells with deionized water (diH_2O) or running buffer.
3. Remove the tape from the bottom of the cassette by pulling the tab across the gel.
4. Insert the cassette into one of the slots in the Criterion cell tank so that the integrated upper buffer chamber faces the center of the cell (**A**).
5. Fill each integrated upper buffer chamber with 60 ml running buffer.
6. Fill each half of the lower buffer tank with 400 ml running buffer to the marked fill line.
7. Load samples using a syringe or a pipet with gel-loading tips.

   Optional: place a sample loading guide on the outer edge of the cassette to help align pipet tips with the wells (this is particularly useful when using multichannel pipets).
8. Place the lid on the tank, aligning the color-coded banana plugs with corresponding jacks on the lid. See Chapters 3–10 for power supply settings.

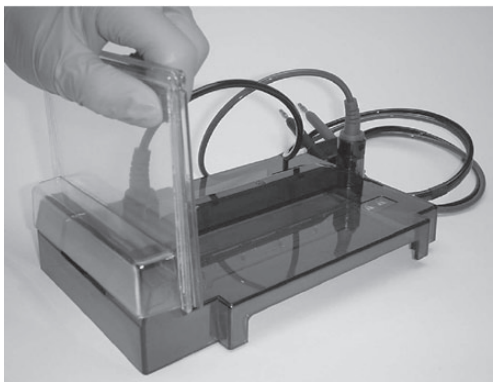
A



2.4 Removing the Gel

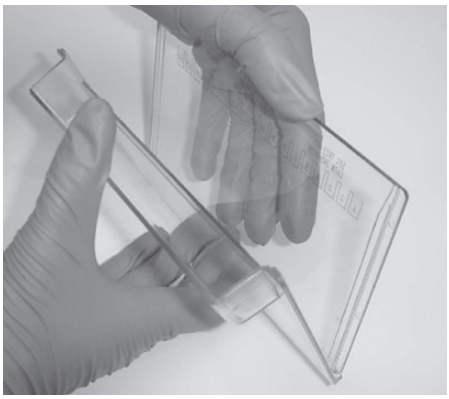
1. After electrophoresis is complete, turn off the power supply and disconnect the electrical leads.
2. Remove the lid from the tank and remove the gel(s) from the cell. Pour off and discard the upper running buffer.
3. Invert the cassette and place the integral buffer chamber over the cassette-opening tool built into the Criterion cell lid (**B**).

B



4. Press down firmly to break the seals on both sides of the cassette. The cassette splits open approximately 1/3 of the way. Alternatively, open the gel cassette by sliding the tapered back of the comb into the slits on either side of the cassette.
5. Pull the two halves of the cassette apart from the top to completely expose the gel (**C**).
6. Remove the gel by either floating the gel into a fixing or staining solution or by carefully lifting the bottom edge of the gel from the cassette.

C



3 SDS-PAGE

3.1 Introduction

Criterion™ precast gels provide versatile systems for the separation of 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 Criterion gels are made without SDS, allowing 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 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 weights 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 usually a thiol reducing agent such as 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 and 3144 for more information.

3.2 Criterion Gel Selection Guide for SDS-PAGE

A number of Criterion gel types are available for SDS-PAGE (Table 3.1) in both single and gradient polyacrylamide percentages. Use the protein migration charts and tables to select the gel type that offers optimum 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, and smaller pore sizes toward the bottom of the gel restrict excessive separation of small molecules

Table 3.1. Criterion precast gels for SDS-PAGE.

Gel Formulation	Gels	Description
TGX (Laemmli-like)	Criterion™ TGX™	Laemmli-like, extended shelf life gels
	Criterion™ TGX Stain-Free™	Laemmli-like, extended shelf life gels with trihalo compounds for rapid fluorescence detection
Tris-HCl (Laemmli)	Criterion Tris-HCl	Tris-HCl Laemmli gels
	Criterion Stain Free™	Tris-HCl Laemmli gels with trihalo compounds for rapid fluorescence detection
Bis-Tris	Criterion™ XT Bis-Tris	Based on a Bis-Tris HCl buffer system (pH 6.4); use these gels with Criterion XT MES buffer for optimum resolution of small proteins
Bis-Tris	Criterion XT Bis-Tris	Based on a Bis-Tris HCl buffer system (pH 6.4); use these gels with Criterion XT MOPS buffer for optimum resolution of midsized proteins
Tris-acetate	Criterion XT Tris-acetate	Based on a Tris-acetate buffer system (pH 7.0)

3.2.1 Criterion TGX and Criterion TGX Stain-Free Gels

Criterion TGX (**T**ris-**G**lycine e**X**tended shelf life) gels are Laemmli-like gels with a proprietary modification that extends their shelf life to 12 months and enhances separation characteristics relative to conventional gel types. The TGX formulation yields Laemmli-like separation patterns with short run times and exceptionally straight lanes and sharp bands. TGX gels offer excellent staining quality, greater transfer efficiency, and molecular weight estimation without the need for special, expensive buffers.

These gels are run using standard Laemmli sample buffer and Tris/glycine/SDS running buffer. Two types of TGX formulations are available:

- Criterion TGX — Laemmli-like, extended shelf life gels
- Criterion TGX Stain-Free — Laemmli-like, extended shelf life gels with trihalo compounds that allow rapid fluorescent detection of proteins with the Criterion Stain Free system, eliminating staining and destaining steps for faster results (see Chapter 5 for more details)

Gel Composition

Crosslinker	2.6% C
Stacking gel	4% T, 2.6% C
Shelf life	~12 months at 2–8°C; expiration date is printed on each cassette

Gel Percentage	Optimum Separation Range
7.5%	40–200 kD
10%	30–150 kD
12%	20–120 kD
18%	10–50 kD
4–15%	20–250 kD
4–20%	10–200 kD
8–16%	20–120 kD
10–20%	10–100 kD
Any kD™ [1]	10–200 kD

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

3.2.2 Criterion Tris-HCl and Criterion Stain Free Gels

These Tris-HCl, Laemmli gels use discontinuous glycinate and chloride ion fronts to form moving boundaries to stack and then separate denatured proteins by size. They are run using standard Laemmli sample buffer and Tris/glycine/SDS running buffer.

- Criterion Tris-HCl — Tris-HCl formulation that offer a shelf life of 12 weeks
- Criterion Stain Free — Tris-HCl gels with unique trihalo compounds that allow rapid fluorescent detection of proteins with the Criterion Stain Free system, eliminating staining and destaining steps for faster results (see Chapter 5 for more details)

Gel Composition

Gel buffer	0.375 M Tris-HCl, pH 8.6
Crosslinker	2.6% C
Stacking gel	4% T, 2.6% C
Storage buffer	0.375 M Tris-HCl, pH 8.6
Shelf life	~12 weeks at 2–8°C; expiration date is printed on each cassette

Gel Percentage	Optimum Separation Range
5%	100–250 kD
7.5%	40–200 kD
10%	30–150 kD
12.5%	20–120 kD
15%	10–100 kD
18%	10–50 kD
4–15%	20–250 kD
4–20%	10–200 kD
8–16%	20–120 kD
10–20%	10–100 kD
10.5–14%	25–200 kD

Migration charts for protein standards on Criterion Tris-HCl, Criterion TGX, and TGX Stain-Free gels.

3.2.3 Criterion XT Bis-Tris Gels

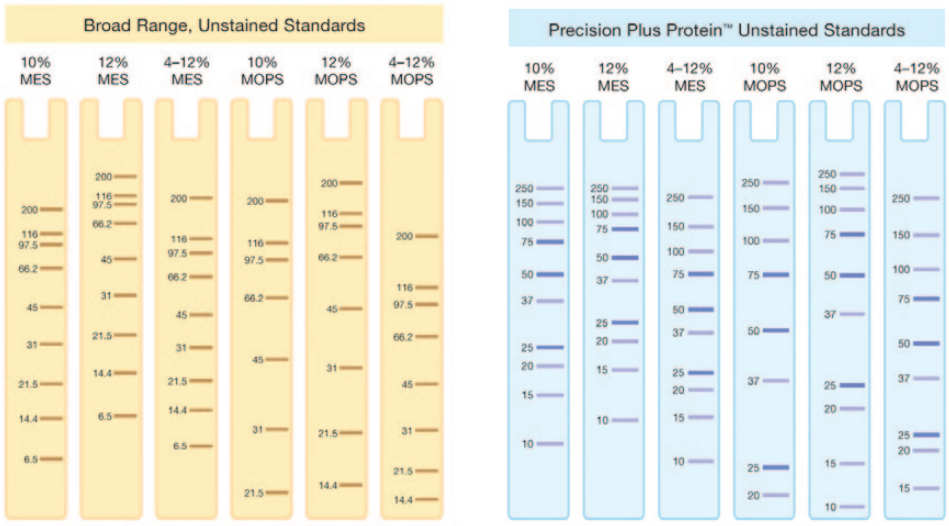
Criterion XT Bis-Tris gels are based on a Bis-Tris HCl buffer system (pH 6.4) that uses discontinuous chloride and MES or MOPS ion fronts to form moving boundaries that stack and separate denatured proteins by size. This chemistry of XT Bis-Tris gels allows maximum stability and consistent results with a shelf life of at least 12 months.

Running XT Bis-Tris gels with either XT MES or XT MOPS denaturing running buffer produces different migration patterns. A combination of these two running buffers and three XT Bis-Tris gels can generate up to six different migration patterns for small to midsize proteins.

Gel Composition

Gel buffer	Bis-Tris HCl, pH 6.4
Crosslinker	5% C
Stacking gel	4% T, 5% C
Storage buffer	Bis-Tris HCl, pH 6.4
Shelf life	12 months at ambient temperature; expiration date is printed on each cassette

Gel Percentage	Optimum Separation Range	
	XT MES Buffer	XT MOPS Buffer
10%	2.5–200 kD	14–220 kD
12%	1–30 kD	6–66 kD
4–12%	2.5–200 kD	10–300 kD



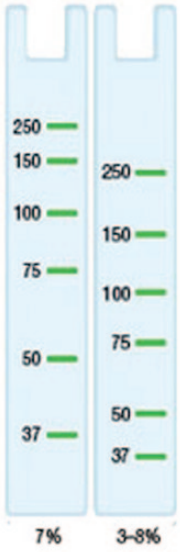
Migration charts for protein standards on Criterion XT Bis-Tris gels.

3.2.4 Criterion XT Tris-Acetate Gels

Criterion XT Tris-acetate gels are based on a Tris-acetate buffer system (pH 7.0). It uses discontinuous acetate and Tricine ion fronts to form moving boundaries that stack and separate large denatured proteins by molecular weight.

Gel Composition	
Gel buffer	Tris-acetate, pH 7.0
Crosslinker	3.8% C
Stacking gel	4% T, 3.8% C
Storage buffer	Tris-acetate, pH 7.0
Shelf life	8 months at 2–8°C; expiration date is printed on each cassette

Gel Percentage	Optimum Separation range
7%	36–200 kD
3–8%	40–400 kD



Migration charts for protein standards on Criterion XT Tris-Acetate gels.

3.3 SDS-PAGE Buffers

Table 3.2. Recommended Criterion precast gels and buffers for SDS-PAGE.

Gel Type	Sample Buffer	Running Buffer
Criterion TGX Criterion TGX Stain-Free Criterion Tris-HCl Criterion Stain Free	Laemmli (catalog #161-0737) Optional: 2-mercaptoethanol (catalog #161-0710) or DTT (catalog #161-0611)	Tris/glycine/SDS (catalog #161-0732)
Criterion XT Bis-Tris	XT sample buffer (catalog #161-0791) Optional: XT reducing agent (catalog #161-0792)	XT MES (catalog #161-0789) XT MOPS (catalog #161-0788)
Criterion XT Tris-acetate		XT Tricine (catalog #161-0790)

3.3.1 Running Buffers

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

Tris/glycine/SDS (1x) (pH 8.3)	25 mM Tris, 192 mM glycine, 0.1% SDS Dilute 100 ml 10x stock (catalog #161-0732) with 900 ml diH_2O
XT MES (pH 6.4)	Dilute 50 ml 20x stock (catalog #161-0789) with 950 ml diH_2O
XT MOPS (pH 6.9)	Dilute 50 ml 20x stock (catalog #161-0788) with 950 ml diH_2O
XT Tricine (pH 8.2)	Dilute 50 ml 20x stock (catalog #161-0790) with 950 ml diH_2O

3.3.2 Sample Buffers

Laemmli	62.5 mM Tris-HCl, pH 6.8, 2% SDS, 25% glycerol, 0.01% (catalog #161-0737) bromophenol blue, 5% β-mercaptoethanol or 100 mM DTT (added fresh)
XT	Use XT sample buffer (catalog #161-0791) and XT reducing agent (catalog #161-0792)

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 12 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.

TGX and Tris-HCl Gels	**XT Gels (Bis-Tris and Tris-Acetate)**
4.75 µl Laemmli sample buffer	5 µl XT sample buffer
0.25 µl β-mercaptoethanol	1 µl XT reducing agent
5 µl sample	1–14 µl sample
10 µl total volume	Make up to 20 µl total volume with diH_2O

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. 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 3.3. Running conditions for SDS-PAGE with Criterion gels in the Criterion cell. Do not run different gel formulations at the same time.

	TGX	Tris-HCl	Bis-Tris		Tris-Acetate
Running buffer	Tris/glycine/SDS	Tris/glycine/SDS	XT MOPS	XT MES	XT Tricine
Standard Conditions					
Power conditions	200 V constant	200 V constant	200 V constant	200 V constant	150 V constant
Expected current (per gel)					
Initial	55–80 mA	90–120 mA	165–175 mA	185–200 mA	170–180 mA
Final	33–43 mA	35–55 mA	60–70 mA	90–110 mA	85–95 mA
Run time	42–45 min	50–55 min	60 min	45 min	65 min
High Voltage (Rapid) Conditions					
Power conditions	300 V constant	—	—	—	—
Expected current (per gel)					
Initial	89–135 mA	—	—	—	—
Final	66–99 mA	—	—	—	—
Run time	20–26 min	—	—	—	—

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 besides 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 allow resolution of proteins with the same molecular weight.

4.2 Criterion™ Gel Selection Guide for Native PAGE

Table 4.1. Criterion precast gels for SDS-PAGE.

Gel Formulation	Gels	Description
Laemmli-like	Criterion™ TGX™	Laemmli-like, extended shelf life gels
	Criterion™ TGX Stain-Free™	Laemmli-like, extended shelf life gels with trihalo compounds for rapid fluorescence detection
Tris-HCl (Laemmli)	Criterion Tris-HCl	Tris-HCl Laemmli gels
	Criterion Stain Free™	Tris-HCl Laemmli gels with trihalo compounds for rapid fluorescence detection
Tris-acetate	Criterion™ XT Tris-acetate	Based on a Tris-acetate buffer system (pH 7.0)

4.2.1 Criterion TGX and Criterion TGX Stain-Free Gels

Criterion TGX (**T**ris-**G**lycine e**X**tended shelf life) gels are Tris-HCl, Laemmli-like gels with a proprietary modification that extends their shelf life to 12 months and enhances separation characteristics relative to conventional gel types. The TGX formulation yields Laemmli-like separation patterns with exceptionally straight lanes and sharp bands and has excellent staining quality and transfer efficiency.

These gels are run using native sample buffer and Tris/glycine running buffer.

Two types of TGX formulations are available:

- Criterion TGX — Laemmli-like gels with the TGX formulation
- Criterion TGX Stain-Free — Laemmli-like, extended shelf life gels that include unique trihalo compounds that allow rapid fluorescent detection of proteins with the Criterion Stain Free system, eliminating staining and destaining steps for faster results (see Chapter 5 for more details)

Gel Composition

Crosslinker	2.6% C
Stacking gel	4% T, 2.6% C
Shelf life	~12 months at 2–8°C; expiration date is printed on each cassette

4.2.2 Criterion Tris-HCl and Criterion Stain Free Gels

These Tris-HCl Laemmli gels are run using native sample buffer and Tris/glycine running buffer.

- Criterion Tris-HCl — Tris-HCl, Laemmli-like formulation that offer a shelf life of 12 weeks
- Criterion Stain Free — Tris-HCl, Laemmli-like gels with unique trihalo compounds that allow rapid fluorescent detection of proteins with the Criterion Stain Free system, eliminating staining and destaining steps for faster results (see Chapter 5 for more details)

Gel Composition

Gel buffer	0.375 M Tris-HCl, pH 8.6
Crosslinker	2.6% C
Stacking gel	4% T, 2.6% C
Storage buffer	0.375 M Tris-HCl, pH 8.6
Shelf life	~12 weeks at 2–8°C; expiration date is printed on each cassette

4.2.3 Criterion XT Tris-Acetate Gels

Criterion XT Tris-acetate gels can also be used to separate proteins by their charge-to-mass ratio (under native PAGE conditions). Separation by native PAGE with XT Tris-acetate gels uses discontinuous acetate and Tricine ion fronts to form moving boundaries to stack and separate proteins by both size and charge.

Gel Composition

Gel buffer	Tris-acetate, pH 7.0
Crosslinker	3.8% C
Stacking gel	4% T, 3.8% C
Storage buffer	Tris-acetate, pH 7.0
Shelf life	8 months at 2–8°C; expiration date is printed on each cassette

4.3 Native PAGE Buffers

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

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% glycerol, 0.01% 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 sample buffer. Only polypeptides with a net negative charge migrate into Criterion 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 protein concentration and load volume of your sample based on the detection method used (see Chapter 12 for approximate stain sensitivities).
2. Dilute the sample in twice the volume of native sample buffer (DO NOT HEAT SAMPLES).

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

Strongly basic proteins (pI >8.5) have a net positive charge and will not enter a Criterion gel under native conditions. To allow polypeptides with a net positive charge to migrate into a native Criterion 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 (see Section 3.5). If high temperature is a concern, run native PAGE at lower voltage; at lower voltages, runs require more time to complete.

Table 4.1. Running conditions for native PAGE for Criterion gels in the Criterion cell.

	Laemmli/Laemmli-like	Tris-Acetate
Running buffer	Native	Native
Power conditions	200 V constant	200 V constant
Expected current (per gel)		
Initial	90–120 mA	70–80 mA
Final	35–55 mA	25–35 mA
Run time	55 min	75 min

5 Criterion Stain Free™ System

5.1 Introduction

The Criterion Stain Free system, which comprises the Gel Doc™ EZ imager, Image Lab™ software, and Criterion™ TGX Stain-Free™ and Criterion Stain Free precast gels, eliminates the time-consuming staining and destaining steps required by other protein detection methods. Criterion TGX (**T**ris-**G**lycine e**X**tended shelf life) Stain-Free gels include a proprietary modification that extends their shelf life to 12 months and enhances separation characteristics relative to conventional gel types. Criterion TGX Stain-Free and Criterion Stain Free gels also include unique trihalo compounds that allow rapid fluorescent detection of proteins with the Gel Doc EZ imager — without staining.

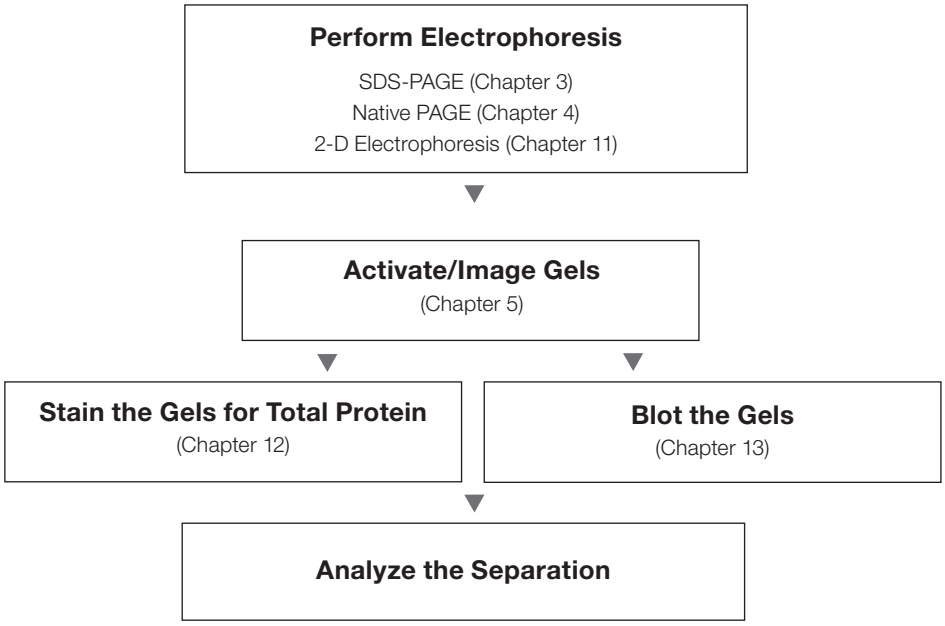
The trihalo compounds in the gels react with tryptophan residues in a UV-induced reaction to produce fluorescence, which can be easily detected by the Gel Doc EZ imager within gels or on low-fluorescence PVDF membranes. Activation of the trihalo compounds in the gels adds 58 Da moieties to available tryptophan residues and is required for protein visualization. Proteins that do not contain tryptophan residues cannot be detected using this system. The sensitivity of the Criterion Stain Free system is comparable to staining with Coomassie Brilliant Blue for proteins with a tryptophan content >1.5%; sensitivity superior to Coomassie staining is possible for proteins with a tryptophan content >3%.

Molecular weights of proteins are estimated by a regression method using Image Lab software. The software generates a standard curve using the molecular weight and relative mobility (R_f) of standard proteins (R_f = distance migrated by the protein/distance migrated by the dye front). The standard curve is then used to estimate the molecular weights of sample proteins.

Benefits of the Criterion Stain Free system include:

- Elimination of staining and destaining steps for faster results
- Automated gel imaging and analysis
- No background variability within a gel or between gels (as is often seen with standard Coomassie staining)
- Reduced organic waste by not requiring acetic acid and methanol for staining or destaining
- Visualization of transferred (blotted) proteins on low-fluorescence PVDF membranes

5.2 Criterion Stain Free Workflow



5.3 Electrophoresis with Criterion TGX Stain-Free Gels

Criterion TGX Stain-Free gels (and Criterion Stain Free gels) are made and packaged without SDS, so they can be used for both SDS and native PAGE applications. To perform electrophoresis with these gels, prepare the sample and running buffers, set up the Criterion cell, and perform the run as directed in Chapters 2–4.

Use unstained standards with Criterion TGX Stain-Free and Criterion Stain Free gels, as some prestained standards are not detected by Stain-Free technology. To monitor electrophoresis, use 10 µl of a 1:1 mixture of Precision Plus Protein™ unstained and Precision Plus Protein All Blue protein standards.

5.4 Using the Gel Doc EZ Imager

Image Criterion TGX Stain-Free and Criterion Stain Free gels and blots in the Gel Doc EZ imager. The imager activates the reaction between the proteins and trihalo compounds in the gel to enable visualization.

- Immediately place the gel in the tray of the imager; no fixation or rinsing steps are required. Prolonged rinsing may diminish image quality and lead to gel deformation
- If desired, stain the gel with any TGX-compatible stains after imaging. Certain stains, if used prior to imaging, eliminate detection capability

Refer to the Gel Doc EZ Stain-Free Sample Tray Instruction Manual (bulletin 10019634) for detailed instructions.

6 Peptide Analysis

6.1 Introduction

Criterion™ Tris-Tricine/peptide 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 that normally interfere with peptide separations to separate completely from peptides. This enables resolution of distinct peptide bands.

6.2 Criterion Tris-Tricine/Peptide 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
Shelf life	~12 weeks at 2–8°C; expiration date is printed on each cassette

6.2.2 Gel Selection Guide

Criterion Tris-Tricine/peptide gels are available in either a single percentage or a linear gradient format.

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

6.3 Tris-Tricine/Peptide 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 100 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 12 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

Power conditions	125 V constant
Starting current	140–150 mA/gel
Final current	60–70 mA/gel
Run time	120 min

7 Isoelectric Focusing (IEF)

7.1 Introduction

Isoelectric focusing (IEF) separates proteins by their net charge rather than molecular weight. Criterion™ IEF gels are cast with Bio-Rad's Bio-Lyte® ampholytes, amphoteric molecules that generate a pH gradient across the gels. Proteins migrate to their isoelectric point (pI), the pH at which the protein has no net charge. Criterion IEF gels contain no denaturing agents, so IEF is performed under native conditions.

7.2 Criterion IEF Gels

7.2.1 Gel Composition

Gel buffer	2% ampholyte, pH 3–10 or 5–8
Crosslinker	3.0% C
Stacking gel	None
Storage buffer	diH_2O
Shelf life	~24 weeks at 2–8°C; expiration date is printed on each cassette

7.2.2 Gel Selection Guide

IEF gel	pH Range
5–8	5–8.0
3–10	4–8.5

7.3 IEF Buffers

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

Running buffers:

IEF cathode buffer (1x)	20 mM lysine (free base), 20 mM arginine (free base) Dilute 100 ml 10x stock (catalog #161-0762) with 900 ml diH_2O
IEF anode buffer (1x)	7 mM phosphoric acid Dilute 100 ml 10x stock (catalog #161-0761) with 900 ml diH_2O
Sample buffer	50% glycerol

7.4 Sample Preparation

1. Determine the appropriate concentration of sample to load (depends on the load volume and the detection method used).
2. Dilute the sample with at least an equivalent volume of sample buffer.

For example, combine:	5 µl sample
	5 µl sample buffer
	10 µl total volume

7.5 Running Conditions

Power conditions (stepwise)	100 V constant 60 min 250 V constant 60 min 500 V constant 30 min
Starting current	5–25 mA/gel
Final current	5–25 mA/gel
Run time	150 min

8 Protease Analysis by Zymogram PAGE

8.1 Introduction

Criterion™ zymogram gels are used to test for proteolytic activity. Gels are cast with gelatin or casein, which acts as a substrate for proteases that are separated in the gel under nonreducing conditions. Proteases are detected by first renaturing the enzymes and then allowing them to break down the substrate. Zymogram gels are stained with Coomassie Brilliant Blue R-250 stain, which stains the substrate while leaving clear areas around active proteases.

8.2 Criterion Zymogram Gels

8.2.1 Gel Composition

Gel buffer	0.375 M Tris-HCl, pH 8.6
Crosslinker	2.6% C
Stacking gel	4% T, 2.6% C
Storage buffer	0.375 M Tris-HCl, pH 8.6, 0.2% NaN_3
Shelf life	~8 weeks at 2–8°C; expiration date is printed on each cassette

8.2.2 Gel Selection Guide

Zymogram Gel	Optimum Separation Range
10% with gelatin	30–150 kD
12.5% with casein	20–120 kD

8.3 Zymogram Buffers

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

Running buffer (1x)	25 mM Tris, 192 mM glycine, 0.1% SDS Dilute 100 ml 10x stock (catalog #161-0732) with 900 ml diH_2O
Sample buffer (catalog #161-0764)	62.5 mM Tris-HCl, pH 6.8, 4% SDS, 25% glycerol, 0.01% Coomassie Brilliant Blue G-250

8.4 Sample Preparation

1. Determine the appropriate protein concentration of your sample based on the detection method and load volume used. (See Chapter 12 for approximate stain sensitivities.)
2. Dilute 1 part sample with 1 part sample buffer. Do not heat the samples.

8.5 Running Conditions

Power conditions	125 V constant
Starting current	90–120 mA/gel
Final current	35–55 mA/gel
Run time	90 min

9 Nondenaturing Nucleic Acid PAGE

9.1 Introduction

Criterion™ 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.

9.2 Criterion TBE Gels

9.2.1 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
Shelf life	~12 weeks at 2–8°C; expiration date is printed on each cassette

9.2.2 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

9.3 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

9.4 Sample Preparation

Determine the DNA concentration of your sample based on the detection method used. (See Chapter 12 for approximate stain sensitivities.) Dilute 4 parts sample with 1 part sample buffer.

9.5 Running Conditions

Table 9.1. Running conditions for nondenaturing nucleic acid PAGE with Criterion gels in the Criterion cell.

	5% and 10% Gels	15% and 4–20% Gels
Power conditions	100 V constant	150 V constant
Expected current (per gel)		
Initial	20–25 mA	27–35 mA
Final	14–18 mA	20–35 mA
Run time	90 min	90 min

10 Denaturing Nucleic Acid PAGE

10.1 Introduction

Criterion™ 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.

10.2 Criterion TBE-Urea Gels

10.2.1 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
Shelf life	~8 weeks at 2–8°C; expiration date is printed on each cassette

10.2.2 Gel Selection Guide

Gel Percentage	Optimum Separation Range
5%	50–1,000 nt
10%	25–300 nt
15%	10–50 nt

10.3 TBE-Urea 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

10.4 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.

10.5 Running Conditions

Table 10.1. Running conditions for denaturing nucleic acid PAGE with Criterion gels in the Criterion cell.

	5% Gels	10% Gels	15% Gels
Power conditions	200 V constant	200 V constant	200 V constant
Expected current (per gel)			
Initial	40–45 mA	20–33 mA	18–22 mA
Final	20–25 mA	14–18 mA	10–15 mA
Run time	90 min	90 min	90 min

11 2-D Electrophoresis

11.1 Introduction

Criterion™ precast gels are available for second-dimension PAGE in 2-D electrophoresis workflows. The IPG-well gels accommodate 11 cm IPG strips. Criterion™ Any kD™ gels are particularly well suited for 2-D electrophoresis applications.

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

- Equilibration of the resolved IPG strips in a reducing buffer containing SDS
- Placing the IPG strip on top of the second-dimension gel

11.2 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 4110009) and buffers in the ReadyPrep™ 2-D starter kit (catalog #163-2105), or other protocols and buffers used with Tris-HCl gels.

11.3 Agarose Overlay

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

- Criterion TGX™ and Tris-HCl gels: prepare 0.5% low-melt agarose (catalog #161-3111), 0.003% bromophenol blue (catalog #161-0404) in 1x Tris/glycine/SDS running buffer. (Alternatively, use ReadyPrep overlay agarose, catalog #163-2111)
- Criterion™ XT gels: prepare 0.5% low-melt agarose (catalog #161-3111), 0.001% bromophenol blue (catalog #161-0404) in appropriate 1x XT running buffer

1. Following equilibration, place the IPG strip, gel side up, on the back plate of the Criterion gel, above the IPG well. The “+” and pH range on the IPG strip should be on the left.
2. 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.
3. Using a disposable pipet, add overlay agarose to 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 backing of the strip to free any trapped bubbles.

11.4 Second-Dimension Electrophoresis

Place the cassettes into the Criterion 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.

12 Detection

12.1 SDS-PAGE and Native PAGE Detection

Following electrophoresis, either stain the gel or use the Criterion Stain Free™ system to visualize proteins in the gel.

- Refer to Table 12.1 for a comparison of total protein stains
- For Criterion™ TGX Stain-Free™ and Criterion Stain Free™ gels, immediately place the gel on the tray of the Gel Doc™ EZ imager; no additional fixation or rinsing steps are required. If desired, stain with any compatible stains (Table 12.1) following imaging. Some stains, if used prior to imaging, can impair imaging quality

Table 12.1. Total protein gel stains for use with Criterion gels.

Stain	Sensitivity (Lower Limit)	Optimum Protein Load (µg/Band)	Advantages	Disadvantages	Imaging	Manual
Criterion 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 transil-lumination	10017295

[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
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
Criterion TGX Stain-Free and Criterion Stain Free Gels						
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 visualization using Criterion Stain Free imaging system	10014472

12.2 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.

12.3 IEF Gel Staining

Samples on IEF gels can be detected using multiple methods. Use Table 12.2 as a guide to selecting an appropriate staining method.

Table 12.2. IEF gel detection methods.

Method	Sensitivity (Lower Limit)	Optimum Protein Load (µg/Band)	Advantages	Disadvantages
Silver Stain Plus kit	0.6–1.2 ng	~0.01	Sensitive, robust, mass spectrometry compatible	Requires TCA fixation
Silver stain	0.6–1.2 ng	~0.01	Stains complex proteins (glyco- or lipoproteins)	Not mass spectrometry compatible
SYPRO Ruby protein gel stain	1.0–10 ng	~0.1	Broad dynamic range, mass spectrometry compatible	Requires laser- or LED-based imaging instrument for maximum sensitivity
Flamingo fluorescent gel stain	0.25–0.5 ng	~0.2	Broad dynamic range, mass spectrometry compatible	Requires laser- or LED-based imaging instrument for maximum sensitivity

12.4 Zymogram Gel Staining

Prior to staining zymogram gels, sample proteases must first be renatured and allowed to break down the substrate contained in the gel. The following protocol provides basic guidelines for detection. Optimal results should be determined empirically.

Renaturing solution	2.5% Triton X-100
Development solution	50 mM Tris, 200 mM NaCl, 5 mM $CaCl_2$ (anhydrous), 0.02% Brij-35 Adjust to pH 7.5
Staining solution	40% methanol, 10% acetic acid, 0.5% Coomassie Blue R-250
Destaining solution	40% methanol, 10% acetic acid

Place gels in renaturing solution for 30 min at room temperature. Incubate gels in development solution at 37ºC for a minimum of 4 hr. Highest sensitivity is typically achieved with overnight incubation. Optimum conditions should be determined empirically. Stain gels with staining solution for at least 1 hr at room temperature. Destain until clear bands appear against the blue background (~30–60 min).

12.5 TBE Gel Staining

Use Table 12.3 as a guide to selecting an appropriate staining method.

Table 12.3. 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

12.6 TBE-Urea Gel Staining

Use Table 12.4 as a guide to selecting an appropriate staining method.

Table 12.4. TBE-urea gel detection methods.

Method	Sensitivity (Lower Limit)	Advantages	Disadvantages
Ethidium bromide	10 ng	Classic fluorescent DNA stain	Carcinogenic
Radiant™ Red	10 ng	Fast, single-step protocol	RNA and ssDNA only
Silver stain	1–2 ng	More sensitive than ethidium bromide	Requires multiple steps

13 Blotting

13.1 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 Criterion™ precast gels onto nitrocellulose or PVDF membranes using either wet or semi-dry transfer techniques.

Assess transfer efficiency using a total protein blot stain such as SYPRO Ruby stain (see Table 12.1). With Criterion™ TGX Stain-Free™ and Criterion Stain Free™ gels, transfer efficiency to low-fluorescence PVDF membranes may also be assessed using the Gel Doc™ EZ imager (see Chapter 5; activate the gel before blotting).

13.2 Transfer

13.2.1 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.

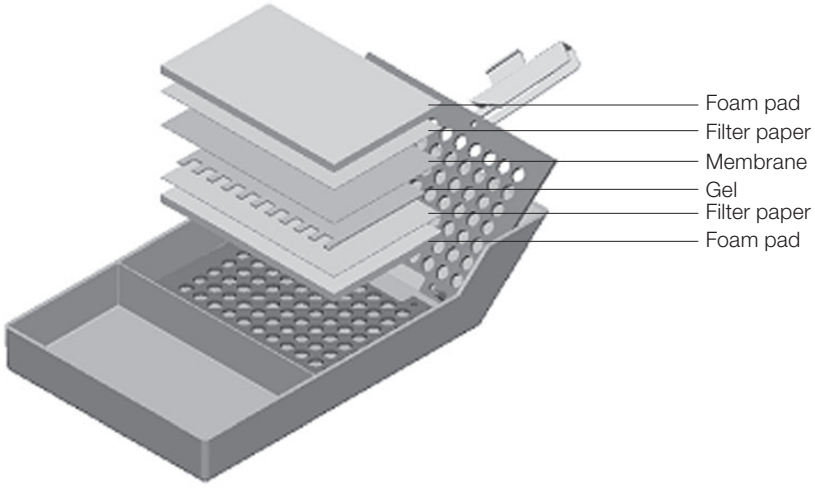
13.2.2 Wet Transfer Using the Criterion Blotter

1. Equilibrate the gels and membranes (for example, in Towbin buffer) for 15 min prior to blot assembly.
2. Assemble the transfer apparatus:
   a) Fill the tank with transfer buffer to ~50% of the fill volume and place a magnetic stir bar inside the tank.
   b) Place the ice block in the pocket in the back of the cell. Flip down the lever to hold the ice block. Alternatively, connect the optional cooling coil to an appropriate recirculating water chiller and place it in the grooves in the back of the tank.
3. Assemble the cassette:
   a) Pour chilled transfer buffer into each compartment of the assembly tray, and then place the membrane (nitrocellulose, PVDF) into the front (small) compartment of the tray to soak.

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

b) Open the cassette and place it in the back (large) compartment of the tray so the red plate with the handle is vertical (anode) and the black plate (cathode) is horizontal and submerged in transfer buffer.

c) Assemble the sandwich as shown, placing the gel closest to the black side of the cassette and the membrane closest to the red plate. Use a blot roller to remove air trapped between the layers of the blot assembly.



Assembly of the gel blot sandwich with the Criterion blotter.

4. Place the assembled cassette into the groove in the tank, aligning the red side of the card with the red electrode. Make sure the magnetic stirbar is free to move. Repeat steps 2–4 for a second blot.
5. Add the remaining transfer buffer to the fill level marked on the tank, place the tank on a stir plate, and begin stirring to maintain even buffer temperature and ion concentration during the transfer.
6. Connect the Criterion blotter to a PowerPac™ HC 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 Criterion Blotter Instruction Manual (bulletin 4006190) or the Protein Blotting Guide (bulletin 2895) for additional information.

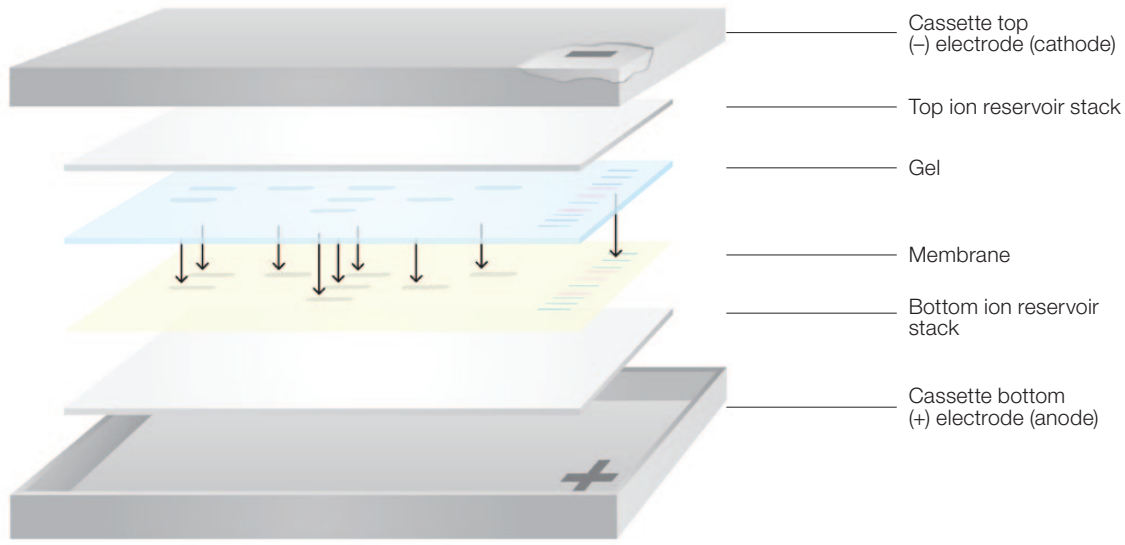
13.2.3 Transfer Using the Trans-Blot® Turbo™ System

1. Open the transfer pack and assemble the components on the cassette in the order shown. For best results, use the roller to remove any air trapped between the layers. If using a single mini or midi sandwich, place the sandwich in the middle of the cassette bottom. With two mini gels, place the sandwiches on a midi stack with the foot of each gel facing the center of the stack.

2. Place the lid on the cassette and lock the lid in place by turning the knob clockwise, using the symbols on the lid as a guide. Slide the cassette into the appropriate bay of the Trans-Blot Turbo cell. Each cassette and bay can hold up to two mini gels or one midi gel (Table 13.1).

3. Start the transfer. With the cassette in the cell, press TURBO and select the gel type. Press A:RUN or B:RUN to begin the transfer. Press LIST to select a Bio-Rad optimized protocol (Table 13.2) or a user-defined protocol. Press NEW to create and run a new protocol.

4. When transfer completes, RUN COMPLETE appears. Pull the cassette straight out of the slot and unlock the lid. Disassemble the blotting sandwich.



Assembly of the gel blot sandwich with the Trans-Blot Turbo system.

Table 13.1. Placement of cassettes in the Trans-Blot Turbo cell.

	Acceptable		Unacceptable	
	Option 1	Option 2	Option 1	Option 2
Upper bay (A)	1 mini gel	2 mini gels -or- 1 midi gel	1 mini gel	2 mini gels -or- 1 midi gel
	-and/or-	-and/or-	-and-	-and-
Lower bay (B)	1 mini gel	2 mini gels -or- 1 midi gel	2 mini gels -or- 1 midi gel	1 mini gel

Table 13.2. Trans-Blot Turbo transfer protocols.

Protocol Name	MW, kD	Time, Min	1 Mini Gel	2 Mini Gels or 1 Midi Gel
STANDARD SD	Any	30	Up to 1.0 A, 25 V constant	Up to 1.0 A, 25 V constant
1.5 MM GEL	Any	10	2.5 A constant, up to 25 V	1.3 A constant, up to 25 V
HIGH MW	>150	10	2.5 A constant, up to 25 V	1.3 A constant, up to 25 V
LOW MW	<30	5	2.5 A constant, up to 25 V	1.3 A constant, up to 25 V
MIXED MW	5–150	7	2.5 A constant, up to 25 V	1.3 A constant, up to 25 V
1 Mini TGX	5–150	3	2.5 A constant, up to 25 V	N/A

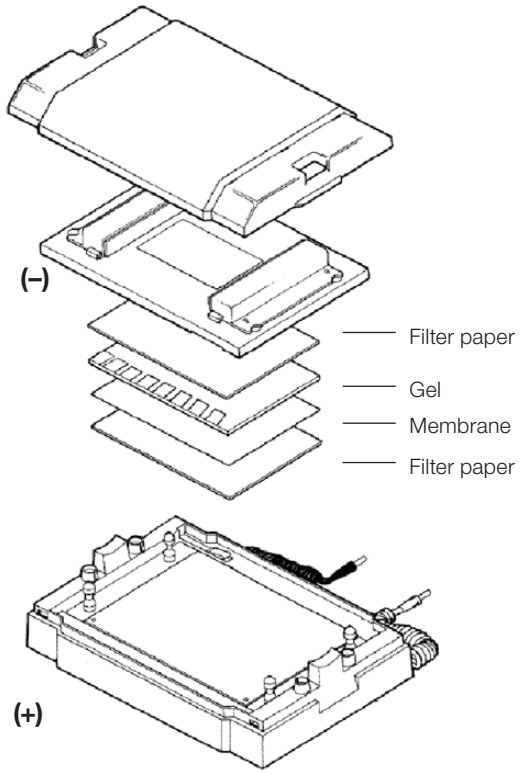
Refer to the Trans-Blot Turbo Instruction Manual (bulletin 10020688) for complete instructions or the Protein Blotting Guide (bulletin 2895) for additional information.

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

1. Equilibrate the gels and membranes (for example, in transfer buffer; see Appendix B for buffer recipes) for 20 min prior to blot assembly.
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 HC power supply and begin transfer at 10–15 V.

For most proteins transferred from Criterion 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 gel blot sandwich with the Trans-Blot SD cell.

13.3 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 13.1. 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	Multi-step 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	Highly sensitive, 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	

To visualize total protein on blots using the Gel Doc EZ imager, refer to Section 5.4.

13.4 Immunodetection

After transfer, blots are ready for downstream processing. While all protein and antibody combinations are different and may require optimization, a general protocol for the immunodetection of a large number of protein and antibody combinations is provided (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). Incubate at room temperature with agitation for 1 hr.
2. Dilute the primary antibody in blocking solution (suggested dilution is specified by the manufacturer). Incubate the blot in the primary antibody solution at room temperature and with agitation for 1 hr.
3. Wash the blot with TTBS as directed in the instructions for the detection method to be used (for example, 5 times, 5 min each at room temperature).
4. Dilute the secondary antibody into TTBS as specified by the manufacturer. Incubate the blot in the secondary antibody solution at room temperature with agitation for 1 hr.
5. Wash the blot with TTBS for 5 min at room temperature with agitation. Pour off the wash solution and repeat 5 times.
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.

14 Troubleshooting

Table 14.1. Troubleshooting electrophoresis and detection with Criterion™ gels. For more troubleshooting tips, refer to the Criterion cell, Criterion blotter, and Trans-Blot® SD cell instruction manuals, or contact Technical Support.

Problem	Cause	Solution
General Troubleshooting Tips		
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 integral buffer chamber	Fill buffer chamber with running buffer
	Insufficient buffer in lower buffer chamber	Fill both halves of lower buffer tank with 400 ml running buffer when running two gels
	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
Bands “smile” across gel: band pattern curves upward at both sides of the gel	Excessive heating of gel	Check buffer composition
		Do not exceed recommended running conditions
	Insufficient buffer	Fill both halves of lower buffer tank with 400 ml running buffer when running two gels
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
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 instructions
	Sample precipitation	Selectively remove predominant proteins in sample
		Dilute sample in sample buffer
	Insoluble materials (for example, cell membranes) in samples	Centrifuge samples to remove particulates prior to sample loading

Problem	Cause	Solution
Artifactual bands at 60–70 kD	Skin keratin contamination	Clean all dishware, and 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
	Not enough running buffer in lower chamber	Add running buffer to fill line of lower buffer chamber
	Diffuse sample loading zone	Load sample with a syringe or gel loading pipet tip
	Sample diffusion during staining	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 sample loading buffers
	Expired gel	Use gels before expiration date printed on the cassette
Criterion™ TGX Stain-Free™ and Criterion Stain Free™ Gels		
Low sensitivity for proteins	Low tryptophan content in proteins	After activation and imaging, stain gel with Bio-Safe™ Coomassie or similar to detect missing bands
Uneven sensitivity or fuzzy bands	Gel was soaked in water or buffer prior to activation and imaging	If possible, activate and image gel using the Gel Doc EZ imager immediately after electrophoresis
Bands are too light or missing from blot (membrane)	Proteins transferred through membrane	Use membrane with smaller pore size
		Decrease transfer time
		Decrease voltage
Standards not visible	Incorrect standards were used	Use unstained standards; some prestained standards are not detected by the Gel Doc EZ imager. To monitor electrophoresis, use a 1:1 mixture of unstained and prestained standards
Dye front at bottom of gels interfering with detection of proteins	Sample constituents present in gel interfering with imaging	Dilute sample in gel running buffer prior to loading
		Activate and image gel, rinse in fixation solution for 30 min, and repeat imaging
High background or low sensitivity on a blot of a Criterion TGX Stain-Free or Criterion Stain Free gel (imaged using the Gel Doc EZ imager)	Low tryptophan content in proteins	After activation and imaging, stain blot with a total protein blot stain to detect missing bands
	Membrane not low-fluorescence PVDF	Always use low-fluorescence PVDF membranes to blot Criterion TGX Stain-Free or Criterion Stain Free gels
	PVDF membrane is dry	Wet PVDF membrane briefly in methanol and wash in water for 1 min before imaging

A Quick Start Guide

The following instructions are for electrophoresis of Criterion™ precast gels using the Criterion system.

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.

Fill the lower buffer chamber with 400 ml running buffer (to the fill mark).

▼

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 Table A.1.

Table A.1. Running conditions for SDS-PAGE in the Criterion cell. Do not run different gel formulations at the same time.

	TGX	Tris-HCl	Bis-Tris		Tris-Acetate
Running buffer	Tris/glycine/SDS	Tris/glycine/SDS	XT MOPS	XT MES	XT Tricine
Standard Conditions					
Power conditions	200 V constant	200 V constant	200 V constant	200 V constant	150 V constant
Expected current (per gel)					
Initial	55–80 mA	90–120 mA	165–175 mA	185–200 mA	170–180 mA
Final	33–43 mA	35–55 mA	60–70 mA	90–110 mA	85–95 mA
Run time	42–45 min	50–55 min	60 min	45 min	65 min
High Voltage (Rapid) Conditions					
Power conditions	300 V constant	—	—	—	—
Expected current (per gel)					
Initial	89–135 mA	—	—	—	—
Final	66–99 mA	—	—	—	—
Run time	20–26 min	—	—	—	—

Table A.2. Standard transfer (blotting) conditions.

Method	Membrane	Time	Voltage
Tank blotting	Nitrocellulose (0.45 µm) PVDF (0.2 µm)	20–60 min	100 V[1]
Semi-dry blotting	Nitrocellulose (0.45 µm) PVDF (0.2 µm)	20–60 min	25 V

[1] For previously optimized protocols for proteins <30 kD, reduce transfer time by 20%.

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% β-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% β-mercaptoethanol (added fresh)

1.0 M Tris-HCl, pH 6.8	6.0 ml
100% Glycerol	12.0 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)
(catalog #161-0768)
Store at 4°C

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

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:

10x Tris/glycine (catalog #161-0734)	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:

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

C Related Literature

Bulletin #	Title
4006183	Criterion™ Cell Instruction Manual
4006197	Criterion™ Dodeca™ Cell Instruction Manual
10014472	Gel Doc™ EZ System Installation Guide
10019634	Stain-Free Sample Tray Instruction Manual
4006190	Criterion Blotter Instruction Manual
4006066	Trans-Blot® SD Semi-Dry Transfer Cell Quick Reference Guide
10020688	Trans-Blot® Turbo™ Blotting System Instruction Manual
1703940	Trans-Blot SD Semi-Dry Transfer Cell Instruction Manual
6007	Criterion™ TGX™ Precast Gels Product Information Sheet
5974	Criterion™ TGX Stain-Free™ Precast Gels Product Information Sheet
2911	Criterion™ XT Precast Gels Product Information Sheet
2895	Protein Blotting Guide
6039	Trans-Blot Turbo Transfer System Brochure
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 Reagents Brochure
2317	Ready-to-Run Buffers and Solutions Brochure
2414	The Little Book of Standards

D Ordering Information

Criterion TGX™ Gels

	12+2 Well (45 µl/well)	18-Well (30 µl/well)	26-Well (15 µl/well)	IPG+1 Well (11 cm IPG Strip)	Prep+2 Well (800 µl/well)
7.5%	567-1023	567-1024	567-1025	567-1021	567-1022
10%	567-1033	567-1034	567-1035	567-1031	567-1032
12%	567-1043	567-1044	567-1045	567-1041	567-1042
18%	567-1073	567-1074	567-1075	567-1071	567-1072
4–15%	567-1083	567-1084	567-1085	567-1081	567-1082
4–20%	567-1093	567-1094	567-1095	567-1091	567-1092
8–16%	567-1103	567-1104	567-1105	567-1101	567-1102
10–20%	567-1113	567-1114	567-1115	567-1111	567-1112
Any kD™	567-1123	567-1124	567-1125	567-1121	567-1192

Criterion™ TGX Stain-Free™ Gels

	12+2 Well (45 µl/well)	18-Well (30 µl/well)	26-Well (15 µl/well)	IPG+1 Well (11 cm IPG Strip)	Prep+2 Well (800 µl/well)
7.5%	567-8023	567-8024	567-8025	567-8021	567-8022
10%	567-8033	567-8034	567-8035	567-8031	567-8032
12%	567-8043	567-8044	567-8045	567-8041	567-8042
18%	567-8073	567-8074	567-8075	567-8071	567-8072
4–15%	567-8083	567-8084	567-8085	567-8081	567-8082
4–20%	567-8093	567-8094	567-8095	567-8091	567-8092
8–16%	567-8103	567-8104	567-8105	567-8101	567-8102
10–20%	567-8113	567-8114	567-8115	567-8111	567-8112
Any kD	567-8123	567-8124	567-8125	567-8121	567-8122

Criterion™ Tris-HCl Gels

	12+2 Well (45 µl/well)	18-Well (30 µl/well)	26-Well (15 µl/well)	IPG+1 Well (11 cm IPG Strip)	Prep+2 Well (800 µl/well)
Criterion Tris-HCl					
5%	345-0001	345-0002	345-0003	—	—
7.5%	345-0005	345-0006	345-0007	—	345-0008
10%	345-0009	345-0010	345-0011	345-0101	345-0012
12.5%	345-0014	345-0015	345-0016	345-0102	345-0017
15%	345-0019	345-0020	345-0021	—	345-0022
18%	345-0023	345-0024	345-0025	—	345-0026
4–15%	345-0027	345-0028	345-0029	345-0103	345-0030
4–20%	345-0032	345-0033	345-0034	345-0104	345-0035
8–16%	345-0037	345-0038	345-0039	345-0105	345-0040
10.5–14%	345-9949	345-9950	345-9951	345-0106	—
10–20%	345-0042	345-0043	345-0044	345-0107	345-0045
Criterion Stain Free™					
10%	345-1012	345-1018	—	—	—
4–20%	345-0412	345-0418	345-0426	—	—
8–16%	345-8162	—	345-8166	345-8161	—

Criterion™ XT Gels

	12+2 Well (45 µl/well)	18-Well (30 µl/well)	26-Well (15 µl/well)	IPG+1 Well (11 cm IPG Strip)	Prep Well (800 µl/well)
Criterion XT Bis-Tris					
10%	345-0111	345-0112	345-0113	345-0115	—
12%	345-0117	345-0118	345-0119	345-0121	345-0120
4–12%	345-0123	345-0124	345-0125	345-0127	345-0126
Criterion XT Tris-Acetate					
7%	345-0135	345-0136	345-0137	—	—
3–8%	345-0129	345-0130	345-0131	345-0133	—

Criterion Tris-Tricine Peptide Gels

	12+2 Well (45 µl/well)	18-Well (30 µl/well)	26-Well (15 µl/well)	Prep+2 Well (800 µl/well)
16.5%	345-0063	345-0064	345-0065	345-0066
10–20%	345-0067	345-0068	345-0069	—

Criterion IEF Gels

	12+2 Well (45 µl/well)	18-Well (30 µl/well)	26-Well (15 µl/well)
pH 3–10	345-0071	345-0072	345-0073
pH 5–8	—	345-0076	—

Criterion Zymogram Gels

	12+2 Well (45 µl/well)	18-Well (30 µl/well)	26-Well (15 µl/well)
10% Zymogram, gelatin	345-0079	345-0080	345-0081
12.5% Zymogram, casein	345-0082	345-0083	345-0084

Criterion TBE Gels

	12+2 Well (45 µl/well)	18-Well (30 µl/well)	26-Well (15 µl/well)
5% TBE	345-0047	345-0048	345-0049
10%	345-0051	345-0052	345-0053
15%	345-0055	345-0056	345-0057
4–20%	345-0059	345-0060	345-0061

Criterion TBE-Urea Gels

	12+2 Well (45 µl/well)	18-Well (30 µl/well)	26-Well (15 µl/well)
5% TBE-urea	—	345-0086	—
10%	345-0088	345-0089	345-0090
15%	345-0091	345-0092	345-0093

Catalog #	Description
Criterion Gel Accessories	
345-9901	Criterion Empty Cassettes, 1.0 mm with 12+2 well comb, 10
345-9902	Criterion Empty Cassettes, 1.0 mm with 18-well comb, 10
345-9903	Criterion Empty Cassettes, 1.0 mm with 26-well comb, 10
345-9904	Criterion Empty Cassettes, 1.0 mm with prep+2 well comb, 10
345-9906	Criterion Empty Cassettes, 1.0 mm with IPG+1 well comb, 10
165-6006	Criterion Sample Loading Guide, 12+2 well, 1
165-6007	Criterion Sample Loading Guide, 18-well, 1
165-6008	Criterion Sample Loading Guide, 26-well, 1
Equipment	
165-6001	Criterion Cell, includes tank, lid with power cables, three sample loading guides
165-4130	Criterion™ Dodeca™ Cell
170-4070	Criterion Blotter with Plate Electrodes
170-4071	Criterion Blotter with Wire Electrodes
170-4155	Trans-Blot® Turbo™ Transfer Starter System
170-3940	Trans-Blot® SD Semi-Dry Electrophoretic Transfer Cell
164-5050	PowerPac™ Basic Power Supply
164-5052	PowerPac HC High Current Power Supply
170-8270	Gel Doc™ EZ Imaging System
170-8274	Stain-Free™ Sample Tray

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 Prestained 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 (2.5–250 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
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
161-0788	XT MOPS Running Buffer, 20x, 500 ml
161-0789	XT MES Running Buffer, 20x, 500 ml
161-0790	XT Tricine Running Buffer, 20x, 500 ml
161-0793	XT MOPS Buffer Kit, includes 500 ml 20x XT MOPS running buffer, 10 ml 4x XT sample buffer, 1 ml 20x XT reducing agent
161-0796	XT MES Buffer Kit, includes 500 ml 20x XT MOPS running buffer, 10 ml 4x XT sample buffer, 1 ml 20 x XT reducing agent
161-0797	XT Tricine Buffer Kit, includes 500 ml 20x XT MOPS running buffer, 10 ml 4x XT sample buffer, 1 ml 20x XT reducing agent
161-0761	10x IEF Anode Buffer, 250 ml
161-0762	10x IEF Cathode Buffer, 250 ml
161-0733	10x Tris/Boric Acid/EDTA, 1 L
161-0770	10x Tris/Boric Acid/EDTA, 5 L
161-0765	Zymogram Renaturation Buffer, 125 ml
161-0766	Zymogram Development Buffer, 125 ml

Catalog #	Description
Premixed Sample Buffers	
161-0737	Laemmli Sample Buffer, 30 ml[1]
161-0738	Native Sample Buffer, 30 ml
161-0791	XT Sample Buffer, 4x, 10 ml
161-0792	XT Reducing Agent, 20x, 1 ml
161-0739	Tricine Sample Buffer, 30 ml
161-0763	IEF Sample Buffer, 30 ml
161-0764	Zymogram Sample Buffer, 30 ml
161-0767	Nucleic Acid Sample Buffer, 5x, 10 ml
161-0768	TBE-Urea Sample Buffer, 30 ml
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 (DTT), 5 g
161-0404	Bromophenol Blue, 10 g
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 Blue R-250 Stain Solution, 1 L
161-0438	Coomassie 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

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

Catalog #	Description
Immunoblot Detection Reagents	
161-0385	Precision Plus Protein™ WesternC™ Pack
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™ Substrate Pack
Blotting Membranes	
162-0232	0.2 µm Nitrocellulose/Filter Paper Sandwich, 8.5 x 13.5 cm, 20 pack
162-0233	0.2 µm Nitrocellulose/Filter Paper Sandwich, 8.5 x 13.5 cm, 50 pack
162-0234	0.45 µm Nitrocellulose/Filter Paper Sandwich, 8.5 x 13.5 cm, 20 pack
162-0235	0.45 µm Nitrocellulose/Filter Paper Sandwich, 8.5 x 13.5 cm, 50 pack
162-0236	Sequi-Blot™ PVDF/Filter Paper Sandwich, 8.5 x 13.5 cm, 20 pack
162-0237	Sequi-Blot PVDF/Filter Paper Sandwich, 8.5 x 13.5 cm, 50 pack
170-4157	Trans-Blot Turbo Midi PVDF Transfer Packs
170-4159	Trans-Blot Turbo Midi Nitrocellulose Transfer Packs

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

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