Ready Gel® Precast Gels

Application Guide

Catalog Number
161-0993

BIO-RAD

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Section 1
General Information

1.1 Introduction

Ready Gel® precast gels greatly simplify polyacrylamide gel electrophoresis. They are specifically for use with the Mini-PROTEAN Systems (Mini-PROTEAN Tetra, Mini-PROTEAN-III and Mini-PROTEAN Dodeca Cells). Stringent production and quality control criteria, and the use of the highest quality reagents, ensure reproducible electrophoretic analysis with minimum effort. Every gel is checked during production for defects, and each lot of gels is further tested by electrophoresis to verify quality.

Ready Gel precast gels come ready to use with preformed sample wells and a stacking gel when necessary. Each Ready Gel Cassette is 8 x 10 cm (H x W) and 4.0 mm thick. Gel dimension is 6.8 x 8.6 cm (HxW) and 1.0 mm thick. Each gel is individually packaged in a leakproof pouch with an absorbent pad containing gel buffer and 0.02% Sodium Azide.

Ready Gel precast gels are available for use in Tris-glycine (Tris-HCl and zymogram gels), Tris-Tricine, TBE, TBE-urea, and IEF buffer systems. The Tris-HCl gels can be used for SDS-PAGE and non-SDS gel electrophoresis. The Tris-Tricine/peptide gels are optimized for peptide electrophoresis. The TBE gels are for use in nucleic acid electrophoresis and can be used for native protein electrophoresis. TBE-urea gels provide denaturing conditions for nucleic acids. Resolution of different size ranges of proteins or nucleic acids can be obtained by choosing the correct gel.

1.2 Mini-format gel System Specifications

Gel material	Polyacrylamide
Gel dimensions	8.6 x 6.8 cm (W x L)
Gel thickness	1.0 mm
Resolving gel height	5.5 cm
Cassette dimensions	10 x 8.0 cm (W x L)
Cassette material	Back (long): acrylic; front (short): glass
Comb material	Polycarbonate
Total running buffer volume	700 ml for 2 gels, 1,000 ml for L. gels (Mini-PROTEAN Tetra Cell)
Storage conditions	Store flat at 4ºC; DO NOT FREEZE

1.3 Ready Gel Comb Configurations

Comb	**Load Volume**
9-well	30 µl
10-well	30 µl
10-well	50 µl
12-well	20 µl
15-well	15 µl
IPG	7 cm ReadyStrip™ IPG strip
Prep	450 µl with one 15 µl reference well

Section 2
Setup and Basic Operation Using Mini-PROTEAN Tetra Cell

2.1 Setting Up and Running Ready Gel Precast Gels

1. Each Ready Gel should be used immediately after it is removed from the storage pouch.
2. Remove the comb and gently rinse the wells with deionized water or running buffer.
3. Use the key knife or a razor blade to cut the tape at the bottom of the gel along the black “cut here” line. It is helpful to cut all the way to the edge of the cassette where the pull tab begins.
4. Pull the tape tab along the cut line, up from the cassette and at an angle towards the comb end of the gel.

Required materials:

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

1. Assembly

Note: When running 2 gels only, use the Electrode Assembly (the one with the banana plugs), Not the Companion Running Module (the one without the banana plugs). When running 4 gels, both the Electrode Assembly and the Companion Running Module must be used, for a total of 4 gels (2 gels per assembly).

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

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

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

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

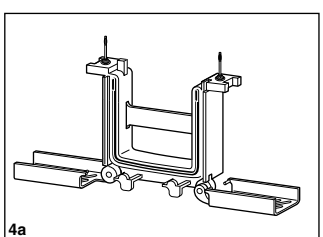
d. While gently squeezing the gel sandwiches or cassettes against the green gaskets with one hand (keeping constant pressure and both gels firmly held in place), slide the green arms of the clamping frame over the gels, locking them into place. Alternatively, you may choose to pick-up the entire assembly with both hands, making sure that the gels do not shift, and simultaneously sliding both arms of the clamping frame into place (see Figure 4c).

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

Note: If running more than 2 gels, repeat steps 1a–d with the Companion Running Module.

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

CAUTION: When running 1 or 2 gels only, DO NOT place the Companion Running Module in the tank. Doing so will cause excessive heat generation and prevent electrophoretic separation.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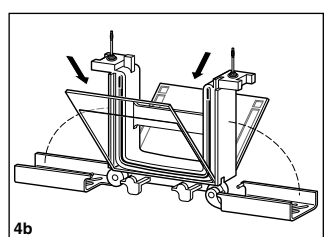
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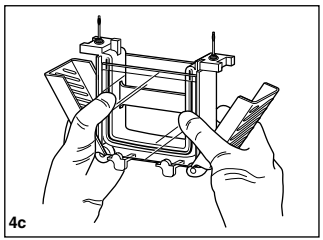

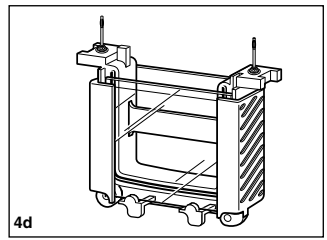

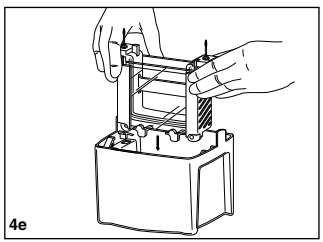

Fig. 4. Assembling the Mini-PROTEAN Tetra Cell Electrophoresis Module.

Section 3
SDS-PAGE

3.1 Introduction

Ready Gel Tris-HCl gels provide a versatile system for the separation of proteins by molecular weight (SDS-PAGE conditions) or charge to mass ratio (native conditions). (See section 4 for native PAGE applications and protocols.) This is possible because Ready Gel Tris-HCl gels are made without SDS, allowing the sample buffer and running buffer to determine the separation mechanism. Historically, SDS-PAGE systems contained SDS in both the gel and the running buffer. Reproducible SDS-PAGE separations are performed in gels lacking SDS provided the sample buffer and running buffers contain sufficient SDS to saturate the proteins during electrophoresis. The recommended concentration of SDS is 2% in sample buffer and 0.1% in running buffers.

SDS-PAGE uses discontinuous chloride and glycine ion fronts to form moving boundaries that stack and then separate SDS-coated polypeptides by molecular weight. Protein samples are prepared in a reducing denaturing sample buffer containing either 2-mercaptoethanol or dithiothreitol as the reducing reagent, and heat and SDS are used to denature the proteins. 2-Mercaptoethanol and dithiothreitol eliminate protein secondary structure by reducing disulfide bonds. SDS minimizes charge variability among proteins, giving them the same charge to mass ratio and forcing them into rod-like shapes. This effectively eliminates the effects of protein conformation and native charge density on the electrophoretic migration distance. Molecular weight determinations are obtained by plotting the logarithm of protein molecular mass vs. the relative mobility (R_f = distance migrated by protein/distance migrated by dye front).

3.2 Ready Gel Tris-HCl Gel Composition

Gel buffer	0.375 M Tris-HCl, pH 8.8
Cross-linker	2.6% C
Stacking gel	4% T, 2.6% C
Storage buffer	0.375 M Tris-HCl, pH 8.8
Shelf life	12 weeks

3.3 Ready Gel Tris-HCl Gel Selection Guide

Tris-HCl gels are available in a wide selection of single percentages and gradients for the separation of proteins by SDS-PAGE.

Tris-HCl Gels	Optimal Separation	Tris-HCl Gradient Gels	Optimal Separation
5%	100–250 kD	4–15%	20–250 kD
7.5%	40–200 kD	4–20%	10–200 kD
10%	30–150 kD	8–16%	20–120 kD
12%	20–120 kD	10–20%	10–100 kD
15%	10–100 kD		
18%	10–50 kD		

3.4 SDS-PAGE Buffers

Running Buffer

1X Working Concentration	10x Stock	
25 mM Tris	Tris base	15.0 g
192 mM glycine	Glycine	72.0 g
0.1% SDS	SDS	5.0 g
	to 500 ml with deionized water	

Note: running buffer should be ~ pH 8.3. Do not adjust the pH.

Sample Buffer

2X Working Concentration	2X Stock	
62.5 mM Tris-HCl, pH 6.8	0.5 M Tris-HCl, pH 6.8	1.0 ml
2% SDS	10% (w/v) SDS	1.6 ml
25% glycerol	Glycerol	2.0 ml
0.01% Bromophenol Blue	1.0% Bromophenol Blue	0.08 ml
5% 2-mercaptoethanol	2-Mercaptoethanol	0.4 ml
or 350 mM DTT (added fresh)	Deionized water	2.92 ml
		8.0 ml

3.5 Sample Preparation

Determine the appropriate protein concentration of your sample based on the detection method and load volume used. (See section 10.1 for approximate stain sensitivities.) Dilute 1 part sample with 1 part sample buffer (see section 3.4) and heat at 95ºC for 5 min.

3.6 Running Conditions

Power conditions	200 V constant	
	Starting current:	50 mA/gel
	Final current:	30 mA/gel
Run time	35 min	

Section 4
Native PAGE

4.1 Introduction

Ready Gel Tris-HCl gels are made without SDS, allowing separation of protein in their native conformation. The nonreducing and nondenaturing environment of native PAGE allows the detection of biological activity and can improve antibody detection. Native PAGE can also be used to resolve multiple protein bands where molecular mass separation by SDS-PAGE would reveal only one.

Native PAGE uses the same discontinuous chloride and glycine ion fronts as SDS-PAGE to form moving boundaries that stack and then separate polypeptides by charge to mass ratio. Proteins are prepared in a nonreducing nondenaturing sample buffer, which maintains the proteins' secondary structure and native charge density. Native PAGE is not suitable for accurate molecular weight determination due to the variability of charge to mass ratio among different proteins.

4.2 Ready Gel Tris-HCl Gel Composition

Gel buffer	0.375 M Tris-HCl, pH 8.8
Cross-linker	2.6% C
Stacking gel	4% T, 2.6% C
Storage buffer	0.375 M Tris-HCl, pH 8.8, NaN_3
Shelf life	12 weeks from the date of manufacture

4.3 Ready Gel Tris-HCl Gel Selection

Native PAGE separates by charge to mass ratio, making individual protein migration protein dependent. Optimal Tris-HCl gel percentages will have to be determined experimentally.

4.4 Native PAGE Buffers

Running Buffer

Working Concentration	10x Stock	
25 mM Tris	Tris base	15.0 g
192 mM glycine	Glycine	72.0 g
	to 500 ml with deionized water	87.0 g

Note: running buffer should be ~ pH 8.3. Do not adjust the pH.

Sample Buffer

2X Working Concentration	2X Stock	
62.5 mM Tris-HCl, pH 6.8	0.5 M Tris-HCl, pH 6.8	1.0 ml
25% glycerol	Glycerol	2.0 ml
1% Bromophenol Blue	1% Bromophenol Blue	1.0 ml
	Deionized water	4.92 ml
		8.0 ml

4.5 Sample Preparation

Determine the desired protein concentration and load volume of your sample based on the detection method used. (See section 10.1 for approximate stain sensitivities). Sample preparation for native PAGE applications requires special consideration. In the absence of SDS, the net charge of a polypeptide will be determined by the pH of the sample buffer. Only polypeptides with a net negative charge will migrate into a native PAGE Tris-HCl gel. Most polypeptides have an acidic or slightly basic pI (~3–8). These proteins can be separated using a standard protocol by diluting 1 part sample with 1 part native sample buffer (see section 4.4; DO NOT HEAT SAMPLES).

Strongly basic peptides (pI >9) will have a net positive charge in a native PAGE Tris-HCl gel. In order for polypeptides with a net positive charge to migrate into a native PAGE Tris-HCl gel, the polarity of the electrodes must be changed by reversing the color-coded jacks when connecting to the power supply.

4.6 Running Conditions

Power conditions	200 V constant	
	Starting current:	50 mA/gel
	Final current:	30 mA/gel
Run time	35 min	

Section 5
Peptide Analysis

5.1 Introduction

Ready Gel Tris-Tricine/peptide gels are optimized for separating peptides and proteins <10 kD. Superior resolution of peptides is achieved by moving the peptide-SDS complexes more slowly through the gel. This allows the faster moving SDS micelles, which normally interfere with peptide separations, to completely separate from the peptides, allowing distinct peptide bands to resolve.

5.2 Ready Gel Tris-Tricine/Peptide Gel Composition

Gel buffer	1.0 M Tris-HCl, pH 8.45
Cross-linker	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 from the date of manufacture

5.3 Ready Gel Tris-Tricine/Peptide Gel Selection Guide

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

Peptide Gel	Optimal Separation
16.5%	15–30 kD
10–20%	1–40 kD

5.4 Tris-Tricine/Peptide Buffers

Running buffer

Working Concentration	10x Stock	
100 mM Tris	Tris base	60.55 g
100 mM Tricine	Tricine	89.60 g
0.1% SDS	SDS	5.0 g
	to 500 ml with deionized water	

Note: Tricine running buffer should be ~ pH 8.25. Do not adjust the pH.

Sample Buffer

Working Concentration	2X Stock	
200 mM Tris-HCl, pH 6.8	1.0 M Tris-HCl, pH 6.8	2.0 ml
2% SDS	10% SDS	2.0 ml
40% glycerol	Glycerol	4.0 ml
0.04% Coomassie Blue G-250	0.5% Coomassie Blue G-250	0.8 ml
2% 2-mercaptoethanol	2-Mercaptoethanol	0.2 ml
or 350 mM DTT (Added fresh)	Deionized water	1.0 ml
		10.0 ml

5.5 Sample Preparation

Determine the appropriate protein concentration of your sample based on the detection method and load volume used. (See section 10.2 for approximate stain sensitivities.) Dilute 1 part sample with 1 part sample buffer and heat at 95ºC for 5 min.

5.6 Running Conditions

Power Conditions

100 V constant
Starting current: 30–35 mA/gel
Final current: 15–20 mA/gel

Run Time

100 min

Section 6
Isoelectric Focusing

6.1 Introduction

Ready Gel IEF gels are cast with Bio-Rad's Bio-Lyte® ampholytes, amphoteric molecules that set up a pH gradient across the gels. Proteins migrate in IEF gels to their neutral isoelectric point (pI), where the protein has zero net charge. Ready Gel IEF gels contain no denaturing agents, so all focusing is performed under native conditions.

6.2 Ready Gel IEF Gel Composition

Gel buffer	2% ampholyte, pH 3–10, 5–8
Cross-linker	3.0% C
Stacking gel	None
Storage buffer	Deionized water, NaN_3
Shelf life	26 weeks from the date of manufacture

6.3 Ready Gel IEF Gel Selection Guide

Ready Gel IEF gels are available in narrow and broad pH ranges.

IEF gel	pH Range
5–8	5–8
3–10	4–8.5

6.4 IEF Buffers

Running buffer

1x Cathode Buffer
20 mM lysine (free base)
20 mM arginine (free base)

5x Cathode Buffer

Lysine (free base)	14.50 g
Arginine (free base)	17.42 g
to 1 L with deionized water	

1x Anode Buffer
7mM% phosphoric acid

10x Anode Buffer

Phosphoric acid	4.2 ml
to 1 L with deionized water	

Sample Buffer 50% glycerol

6.5 Sample Preparation

Determine the appropriate protein concentration of your sample based on the detection method and load volume used. (See section 10.3 for approximate stain sensitivities.) Dilute 1 part sample with 1 part sample buffer.

6.6 Running Conditions

Power conditions

Stepwise

100 V constant	60 min
250 V constant	60 min
500 V constant	30 min
Starting current:	5–15 mA/gel
Final current:	5–15 mA/gel

Run time 150 min

Section 7
Protease Analysis by Zymogram PAGE

7.1 Introduction

Ready Gel zymogram gels are used to test for proteolytic activity when performing protein characterizations. Gels are cast with gelatin or casein, which act as substrates for proteases that are separated on the gel. Proteases are detected by renaturing the enzyme followed by a development period in which the protease breaks down the substrate. Zymogram gels are stained with Coomassie Blue R-250, which stains the substrate while leaving clear areas around active proteases.

7.2 Ready Gel Zymogram Gel Composition

Gel buffer	0.375 M Tris-HCl, pH 8.6
Cross-linker	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	12 weeks from the date of manufacture

7.3 Ready Gel Zymogram Gel Selection Guide

Ready Gel zymogram gels are available with either gelatin or casein as substrate and should be selected based on their substrate and separation range.

Zymogram Gel	Optimal Separation
10% zymogram gel with gelatin	30–150 kD
12% zymogram gel with casein	20–120 kD

7.4 Zymogram Buffers

Running buffer

Working Concentration	10x Stock	
25 mM Tris	Tris base	15.0 g
192 mM glycine	Glycine	72.0 g
0.1% SDS	SDS	5.0 g
	to 500 ml with deionized water	

Note: running buffer should be ~pH 8.3. Do not adjust the pH.

Sample Buffer

Working Concentration	2X Stock	
62.5 mM Tris-HCl, pH 6.8	0.5 M Tris-HCl, pH 6.8	1.25 ml
4% SDS	10% SDS	4.0 ml
25% glycerol	Glycerol	2.5 ml
0.01% Bromophenol Blue	1% Bromophenol Blue	0.1 ml
	Deionized water	2.15 ml
		10 ml

7.5 Sample Preparation

Determine the appropriate protein concentration of your sample based on the detection method and load volume used. (See section 10.4 for approximate stain sensitivities.) Dilute 1 part sample buffer with 1 part Zymogram sample buffer. Dry samples can be dissolved directly in sample buffer. Do not heat.

7.6 Running Conditions

Power conditions

100 V constant
Starting current: 10–15 mA/gel
Final current: 6 mA/gel

Run time

90 min

Section 8
Nondenaturing Nucleic Acid PAGE

8.1 Introduction

Ready Gel TBE gels are ideal for separating small dsDNA fragments, especially PCR products. The uniform nature of DNA molecules provides samples with near-uniform charge to mass ratio, allowing nondenaturing nucleic acid PAGE to separate dsDNA by mass using a continuous TBE buffer system.

8.2 Ready Gel TBE Gel Composition

Gel buffer	89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3
Cross-linker	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 from the date of manufacture

8.3 Ready Gel TBE Gel Selection Guide

Ready Gel TBE gels are available in a selection of single percentages and gradients for the separation of dsDNA.

TBE Gels	Optimal Separation	TBE Gradient Gels	Optimal Separation
5%	200–2,000 bp	4–20%	10–2,000 bp
10%	50–1,500 bp		
15%	20–1,000 bp		

8.4 Nondenaturing Nucleic Acid PAGE Buffers

Running Buffer

Working Concentration	10x Stock	
50 mM Tris	Tris base	0.06 g
89 mM boric acid	Boric acid	27.5 g
5 mM EDTA	0.5 M EDTA (pH 8.0)	0.1 ml
	to 500 ml with deionized water	

Note: TBE running buffer should be ~ pH 8.3. Do not adjust the pH.

Sample Buffer

2X Working Concentration		
50 nM EDTA	Tris Base	0.06 g
25% glycerol	0.5 M EDTA	0.1 ml
0.2% Bromophenol Blue	Glycerol	2.5 ml
0.2% Xylene Cyanole FF	1% Bromophenol Blue	2.0 ml
	1% Xylene Cyanole FF	2.0 ml
	Make up to 10 ml with deionized water	

8.5 Sample Preparation

Determine the desired DNA concentration of your sample based on the detection method used. (See section 10.5 for approximate stain sensitivities.) Dilute 1 part sample with 4 parts sample buffer (see section 8.4).

8.6 Running Conditions

Power conditions

100 V constant	
Starting current:	13 mA/gel
Final current:	11 mA/gel

Run time 45–105 min

Section 9
Denaturing Nucleic Acid PAGE

9.1 Introduction

Ready Gel TBE-urea gels are ideal for separating small ssDNA and RNA fragments. Applications include oligonucleotide analysis, RNase protection assays, and northern blotting.

9.2 Ready Gel TBE-Urea Gel Composition

Gel buffer	89 mM Tris, 89 mM boric acid, 2 mM EDTA, 7 M urea, pH 8.3
Cross-linker	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 from the date of manufacture

9.3 Ready Gel TBE-Urea Gel Selection Guide

Ready Gel TBE-urea gels are available in a range of single percentage gels.

TBE-Urea	Optimal Separation
5%	50–1,000 bases
10%	25–300 bases
15%	10–50 bases

9.4 TBE-Urea Buffers

Running Buffer

Working Concentration	10x Stock	
89 mM Tris	Tris base	54.0 g
89 mM boric acid	Boric acid	27.5
2 mM EDTA	0.5 M EDTA (pH 8.0)	20.0 ml
	to 500 ml with deionized water	

Sample Buffer

Working Concentration		
89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0	10x TBE	1.0 ml
12% Ficoll	Ficoll	1.2 g
0.01% Bromophenol Blue	Urea	4.2 g
0.02% Xylene Cyanole FF	1% Bromophenol blue	0.1 ml
7 M urea	1% Xylene Cyanole FF	0.2 ml
	0.5 M EDTA	0.02 ml
	Make up to 10 ml with deionized water	

9.5 Sample Preparation

Determine the desired ssDNA or RNA concentration for your sample based on the detection method used. (See section 10.6 for appropriate stain sensitivities.) Dilute 1 part sample with 1 part TBE-urea sample buffer. Dry samples can be dissolved directly in sample buffer. Heat to 70–90°C 4 min before loading.

9.6 Running Conditions

Power conditions

200 V constant
Starting current: 15 mA/gel
Final current: 10 mA/gel

Run time

40–70 min

Section 10
Detection

10.1 SDS-PAGE and Native PAGE Detection

Total Protein Gel Stains

Method	Sensitivity	Optimal Protein Load	Advantages	Disadvantages
Coomassie Blue R-250	36–47 ng	~0.5 µg/band	Laboratory standard	Requires MeOH
Bio-Safe™ Coomassie stain	8–28 ng	~0.5 µg/band	Nonhazardous, uses no MeOH	More steps than Coomassie Blue R-250
Copper stain	6–12 ng	~0.2 µg/band	Fast, reversible stain be photographed; SDS-PAGE only	Negative stain, must
Zinc stain	6–12 ng	~0.2 µg/band	High-contrast, fast, reversible stain SDS-PAGE only	Negative stain, must be photographed;
Silver Stain™ Plus kit	0.6–1.2 ng	~0.01 µg/band	Simple, robust, mass spectrometry compatible	Will not stain glycoproteins
Silver stain	0.6–1.2 ng	~0.01 µg/band	Stains complex proteins, i.e., glycoproteins and lipoproteins	Not mass spectrometry compatible
SYPRO Orange protein stain	4–8 ng	~0.2 µg/band	Will not stain nucleic acids; mass spectrometry compatible	Optimization required for maximum sensitivity
SYPRO Ruby protein gel stain	1–10 ng	~0.2 µg/band	Broad dynamic range, simple robust protocol maximum sensitivity	Requires imaging instrument for
Flamingo Fluorescent gel stain	0.25–0.5 ng	~0,2 ng/band	Broad dynamic range mass spec compatible	Requires imaging instrument for maximum sensitivity

Total Protein Blot Stains

Method	Sensitivity	Optimal Protein Load	Advantages	Disadvantages
SYPRO Ruby protein blot stain	2–8 ng	~0.2 µg/band immunological procedures	Compatible with mass spectrometry, Edman-based sequencing, and standard maximum sensitivity	Multiple step protocol; Requires imaging instrument for
Colloidal gold stain	1 ng	~0.1 µg/band	Sensitive, one step	Not compatible with nylon membranes
Enhanced colloidal gold detection kit	10–100 pg	~0.1 µg/band	Increases sensitivity of colloidal gold stain	Multiple steps
AmidoBlack 10B	100–1,000 ng	~5 µg/band	Standard membrane stain, economical	Low sensitivity

Immunoblot Detection

Method	Sensitivity	Optimal Protein Load	Advantages	Disadvantages
4CN colorimetric (HRP)	500 pg	~0.25 µg/band	Fast detection	Results may fade
DAB colorimetric (HRP)	500 pg	~0.25 µg/band	Fast detection	Contains toxic chemicals
Opti-4CN colorimetric (HRP)	100 pg	~0.05 µg/band	Color does not fade	More expensive than 4CN
Amplified Opti-4CN colorimetric (HRP)	10 pg	~0.005 µg/band	High sensitivity, low background	Amplification requires additional steps
BCIP/NBT colorimetric (AP)	100 pg	~0.05 µg/band	Sensitive, multiple antigen	May detect endogenous enzyme activity
Amplified alkaline phosphatase	10 pg	~0.005 µg/band	High sensitivity	Amplification requires additional steps
Immun-Star™ chemiluminescent (AP)	10 pg	~0.005 µg/band	Long-lasting signal, short and multiple exposures possible	Requires visualization on film or instrumentation
Immun-Star™ chemiluminescent (hrp)	1–3 pg	~0.005 µgband	Intensifies signal output, very sensitive	Requires visualization on film or instrumentation
Immun-Star WesternC (HRP)	10 fg	~0.005 µgband	long-lasting signal short and multiple exposures possible	Requires visualzation on on film or instrumentation

10.2 Peptide Gel Staining

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

Fixative Solution

40% methanol
10% acetic acid

Coomassie Brilliant Blue G-250 Stain Solution

0.025% 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 Coomassie Brilliant Blue G-250 stain solution for 1 hr. Stain should only be used once. Reuse of stain could result in loss of sensitivity. Destain gels 3 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.

10.3 IEF Gel Staining

Samples on IEF gels can be detected using multiple methods. Use the following table as a guide to select an appropriate staining method.

Method	Sensitivity	Optimal Protein Load	Advantages	Disadvantages
IEF stain	40–50 ng	~0.5 µg/band	Simple, no fixation required	Requires MeOH
Silver Stain Plus kit	0.6–1.2 ng	~0.01 µg/band	Simple, robust, mass spectrometry compatible	Requires TCA fixation
Silver stain	0.6–1.2 ng	~0.01 µg/band	Stains complex proteins, i.e. glycoproteins and lipoproteins	Requires TCA fixation

10.4 Zymogram Gel Staining

Prior to staining zymogram gels, sample proteases must be first 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

Proteins must be renatured first by placing the 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. Optimal results should be determined empirically. Stain gels with Coomassie Brilliant Blue R-250 staining solution for at least 1 hr at room temperature. Destain until clear bands appear against the blue background, approximately

30–60 min.

10.5 TBE Gel Staining

Use the following table as a guide to select an appropriate staining method.

Method	Sensitivity	Optimal Protein Load	Advantages	Disadvantages
Ethidium bromide	50 ng	~0.10 µg/band	Classic fluorescent DNA stain	Carcinogenic
Silver stain	1.0–2.0 ng	~0.5 µg/band	More sensitive than ethidium bromide	Multiple steps

10.6 TBE-Urea Gel Staining

Samples on denaturing nucleic acid gels can be detected using multiple methods. Use the following table as a guide to select an appropriate staining method.

Method	Sensitivity	Optimal Protein Load	Advantages	Disadvantages
Ethidium bromide	50 ng	~0.10 µg/band	Classic fluorescent DNA stain	Carcinogenic
Radiant® Red	10 ng	~0.10 µg/band	Fast single-step protocol	RNA and ssDNA only
Silver stain	1.0–2.0 ng	~0.5 µg/band	More sensitive than ethidium bromide	Multiple steps

Section 11
Stock and Staining Solutions

11.1 Stock Solutions

0.5 M Tris-HCl, pH 6.8

6.06 g Tris base
~60 ml deionized water
Adjust to pH 6.8 with HCl. Make to 100 ml with deionized water and store at 4°C.

10% SDS

Dissolve 1 g SDS in water with gentle stirring and bring to 10 ml with deionized water.

1% Bromophenol Blue

Dissolve 0.1 g of Bromophenol Blue in 10 ml deionized water with gentle stirring.

1% Xylene Cyanole FF

Dissolve 0.1 g of Xylene Cyanole FF in 10 ml deionized water with gentle stirring.

0.5 M EDTA

18.6 g of EDTA
~ 50 ml of deionized water
Adjust to pH 8.0 with 1 N NaOH. Make to 100 ml with deionized water and store at 4°C.

11.2 Protein Staining Solutions

Coomassie Blue R-250 Staining Solution (0.1%)

	Final Concentration	
Methanol	400 ml	40%
Acetic acid	100 ml	10%
Coomassie Blue R-250	1.0 g	0.1%
Deionized water	500 ml	

Dissolve Coomassie R-250 in methanol/acetic acid. Add deionized water to a final volume of 500 ml.

Coomassie Blue R-250 Destaining Solution

	Final Concentration	
Methanol	400 ml	40%
Acetic acid	100 ml	10%
Deionized water	500 ml	

Silver Staining

See Bio-Rad's silver stain (catalog #161-0443) or Silver Stain Plus kit (catalog #161-0449) instructions.

IEF Staining Solution

	Final Concentration	
Isopropyl alcohol	270 ml	27%
Acetic acid	100 ml	10%
Coomassie Blue R-250	0.4 g	0.04%
Crocein Scarlet	0.5 g	0.05%
Deionized water	630 ml	

IEF Destaining Solution

	Final Concentration	
Methanol	400 ml	40%
Acetic acid	100 ml	10%
Deionized water	500 ml	

11.3 Peptide Staining Solutions

Fixative Solution

	Final Concentration	
Methanol	400 ml	40%
Acetic acid	100 ml	10%
Deionized water	500 ml	

Coomassie Blue G-250 Staining Solution (0.025%)

	Final Concentration	
Acetic acid	100 ml	10%
Coomassie Blue G-250	0.25 g	0.025%
Deionized water	900 ml	

Coomassie Blue G-250 Destaining Solution

	Final Concentration	
Acetic acid	100 ml	10%
Deionized water	900 ml	

11.4 Zymogram Staining Solutions

Renaturation Buffer

	Final Concentration	
Triton X-100	25 g	2.5 %
Deionized water	900 ml	
to 1 L with deionized water		

Development Solution

	Final Concentration	
Tris base	6.06 g	50 mM
NaCl	11.7 g	200 mM
$CaCl_2$ (anhydrous)	0.56 g	5 mM
30% Brij-35	0.67 ml	0.02%

Dissolve in 900 ml deionized water, adjust to 7.5 with 6 N HCl, make to 1 L with deionized water.

Staining Solution

	Final Concentration	
Methanol	400 ml	40%
Acetic acid	100 ml	10%
Coomassie Blue R-250	5 g	0.5%
Deionized water	500 ml	

Dissolve Commassie R-250 in Methnaol/acetic acid. Add deionized water to final volume of 500 ml.

11.5 Nucleic Acid Staining Solutions

Ethidium Bromide Staining

Use Bio-Rad's ethidium bromide tablets or ethidium bromide solutions (catalog #161-0430 or 161-0443) for nucleic acid staining solutions.

Silver Staining

See instructions for Bio-Rad's silver stain, catalog #161-0443, or Silver Stain Plus kit, catalog #161-0449.

Radiant® Red Staining

Use Radiant Red stain, catalog #170-3122, for RNA staining.

Section 12
Troubleshooting

Improper storage of Ready Gel precast gels can produce numerous artifacts. Gels should be stored flat at 4ºC. Avoid freezing or prolonged storage above 4ºC. If you suspect your gels have been stored improperly, DO NOT USE THEM.

Problem	Possible Cause	Solution
Samples do not migrate into gel	Tape at the bottom of the cassette not removed	Remove tape
	Insufficient buffer in integral buffer chamber	Fill buffer chamber with 125 ml running buffer
	Insufficient lower electrode buffer	Fill both halves of the lower buffer tank with sufficient running buffer
	Electrical disconnection	Check electrodes and connections
Bands “smile” across gel, band pattern curves upward at both sides of the gel	Excess heating of gel	Check buffer composition
		Completely fill both halves of the lower buffer tank with sufficient running buffer
		Do not exceed recommended running conditions
Skewed or distorted bands, lateral band spreading	Excess salt in samples	Remove salts from sample by dialysis or desalting column prior to sample preparation
	Insufficient sample buffer or wrong formulation	Check buffer composition and dilution instructions
Problem	**Possible Cause**	**Solution**
Vertical streaking	Overloaded samples	Dilute sample

		Selectively remove predominant protein in the sample
	Sample precipitation	Centrifuge samples to remove particulates prior to sample loading
Gels run too fast, provide poor resolution, and gel temperature is too high	Running buffer is too concentrated	Check buffer composition
Artifact bands at ~60–70 kD	Possible skin keratin contamination	Clean all dishware and wear gloves while handling and loading gel
		Filter all solutions through nitrocellulose
		Use 10% iodoacetamide to eliminate keratin bands

Section 13
Ordering Information

13.1 Ready Gel Precast Gels

Ready Gel Tris-HCl Gels	10-Well 30 µl	15-Well 15 µl	Prep Well 450 µl	10-Well 50 µl	12-Well 20 µl	9-Well 30 µl	IPG Comb 7 cm IPG Strip
5% Tris-HCl	161-1210	161-1211		161-1213	161-1214		
7.5% Tris-HCl	161-1100	161-1118	161-1136	161-1154	161-1172		
10% Tris-HCl	161-1101	161-1119	161-1137	161-1155	161-1173	161-1191	161-1390
12% Tris-HCl	161-1102	161-1120	161-1138	161-1156	161-1174		161-1391
15% Tris-HCl	161-1103	161-1121	161-1139	161-1157	161-1175		
18% Tris-HCl	161-1216	161-1217		161-1219	161-1220		
4–15% Tris-HCl	161-1104	161-1122	161-1140	161-1158	161-1176	161-1194	161-1392
4–20% Tris-HCl	161-1105	161-1123	161-1141	161-1159	161-1177		161-1393
8–16% Tris-HCl	161-1222	161-1223		161-1225	161-1226		161-1394
10–20% Tris-HCl	161-1106	161-1124	161-1142	161-1160	161-1178		161-1395
Ready Gel TBE Precast Gels							
5%, TBE	161-1109	161-1127	—	161-1163	161-1181		
10%, TBE	161-1110	161-1128	—	161-1164	161-1182		
15%,TBE	161-1228	161-1229	—		161-1232		
4–20%, TBE	161-1234	161-1235	—	161-1237			
Ready Gel Tris-Tricine/Peptide Precast Gels							
16.5% Tris-Tricine/Peptide	161-1107	161-1125	161-1143	161-1161	161-1179	161-1197	
10–20% Tris-Tricine/Peptide	161-1108	161-1126	161-1144	161-1162	161-1180	161-1198	

Ready Gel IEF Precast Gels

IEF pH 3–10	161-1111	161-1129		161-1165	
IEF pH 5–8	161-1112				

Ready Gel Zymogram Precast Gels

10% Zymogram, gelatin	161-1113	161-1131	—	161-1167	161-1185
12.5% Zymogram, casein	161-1114		—	161-1168	

Ready Gel TBE-Urea Precast Gels

5% TBE-Urea	161-1115	161-1133		—	
10% TBE-Urea	161-1116	161-1134			
15% TBE-Urea	161-1117	161-1135			161-1189

13.2 Buffers

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
161-0771	10x Tris/Glycine, 5 L
161-0744	10x Tris/Tricine/SDS, 1 L
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

Individual Reagents

161-0719	Tris, 1 kg
161-0716	Tris, 500 g
161-0717	Glycine, 250 g
161-0718	Glycine, 1 kg
161-0724	Glycine, 2 kg
161-0301	SDS, 100 g
161-0710	2-Mercaptoethanol, 25 ml
161-0610	Dithiothreitol, 1 g
161-0611	Dithiothreitol, 5 g
161-0404	Bromophenol Blue, 10 g

Premixed Sample Buffers

161-0737	Laemmli Sample Buffer, 30 ml*
161-0738	Native Sample Buffer, 30 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

* Requires addition of 2-mercaptoethanol or DTT

13.3 Detection Reagents

Total Protein Gel Stains

161-0436	Coomassie Blue R-250 Stain Solution, 1 L
161-0438	Coomassie Blue R-250 Destain Solution, 1 L
161-0400	Coomassie Brilliant Blue R-250, 10 g
161-0786	Bio-Safe Coomassie Stain, 1 L
161-0470	Copper Stain and Destain Kit
161-0440	Zinc Stain and Destain Kit
161-0449	Silver Stain Plus Kit
161-0443	Bio-Rad Silver Stain Kit
170-3120	SYPRO Orange Protein Stain, 500 µl
170-3125	SYPRO Ruby Protein Gel Stain, 1 L
161-0490	Flamingo Fluorescent Gel Stain (10x), 20 ml
161-0491	Flamingo Fluorescent Gel Stain (10x), 100 ml
161-0492	Flamingo Fluorescent Gel Stain (10x), 500 ml

Immunoblot Detection

170-6431	HRP Conjugate Substrate Kit, 4CN
170-6535	HRP Color Development Reagent, DAB
170-8238	Amplified Opti-4CN Kit
170-8235	Opti-4CN Substrate Kit
170-6432	BCIP/NBT AP Conjugate Substrate Kit
170-6412	Amplified Alkaline Phosphatase Kit
170-5012	Immun-Star™ Substrate Pack

Total Protein Blot Stains

170-3127	SYPRO Ruby Protein Blot Stain, 200 ml
170-6527	Colloidal Gold Total Protein Stain, 500 ml
170-6517	Enhanced Colloidal Gold Detection Kit
161-0402	Amido Black 10B, 25 g

13.4 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

13.5 Protein and DNA Standards

161-0363	Precision Plus Protein™ Unstained Standards (10–250 kD), 1,500 µl, 150 applications
161-0373	Precision All Blue Prestained Standards (10–250 kD), 500 µl, 50 applications
161-0324	Kaleidoscope™ Prestained Standards, 500 µl, 50 applications
161-0326	Polypeptide SDS-PAGE Standards (1.4–26.6 kD), 200 µl, 400 applications
161-0310	IEF Standards, pI range 4.45–9.6, 250 µl, 500 applications
161-0375	Precision Plus Protein™ Kalaidoscope Standards 500 µl, 50 applications
161-0370	Precision Plus Protein™ WesternC Standards, 250 µl, 50 applications
170-8351	20 bp EZ Load™ Molecular Ruler (20–1,000 bp), 50 µg, 100 applications
170-8352	100 bp EZ Load Molecular Ruler (100–1,000 bp), 25 µg, 100 applications
170-8353	100 bp PCR EZ Load Molecular Ruler (100–3,000 bp), 40 µg, 100 applications
170-8200	AmpliSize® Molecular Ruler (50–2,000 bp), 25 µg, 50 applications
165-8004	Mini-PROTEAN Tetra Cell for Ready Gel Precast gels

13.6 Equipment

165-3302	Mini-PROTEAN 3 Electrophoresis Module
170-3930	Mini Trans-Blot® Electrophoresis Transfer Cell

Catalog Number
161-0993

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Bio-Rad Laboratories, Inc.

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Web site *www.bio-rad.com* ***USA*** *(800) 4BIORAD* ***Australia*** *02 9914 2800*
Austria *(01) 877 89 01* ***Belgium*** *09-385 55 11* ***Brazil*** *55 21 507 6191*
Canada *(905) 712-2771* ***China*** *(86-21) 63052255*
Czech Republic *(420) 2-4141 0532* ***Denmark*** *45 44 52-1000*
Finland *358 (0)9 804 2200* ***France*** *01 47 95 69 65* ***Germany*** *089 318 84-177*
Hong Kong *852-2789-3300* ***India*** *(91-124) 6398112/113/114, 6450092/93*
Israel *03 951 4127* ***Italy*** *39 02 216091* ***Japan*** *03-5811-6270*
Korea *82-2-3473-4460* ***Latin America*** *305-894-5950*
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South Africa *00 27 11 4428508* ***Spain*** *34 91 590 5200*
Sweden *46 (0)8-55 51 27 00* ***Switzerland*** *061 717-9555* ***United Kingdom*** *0800-181134*

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