

## ELECTROPHORESIS

# Mini-PROTEAN<sup>®</sup> and Criterion<sup>™</sup> TGX Stain-Free<sup>™</sup> Precast Gels

### Key Benefits:

- Complete protein separations, gel imaging, and data analysis in as little as 20 min
- Run times as short as 15 min
- Transfer in as little as 3 min with the Trans Blot<sup>®</sup> Turbo<sup>™</sup> system and in 15 min using tank blotting
- Inexpensive Laemmli buffer system, low running costs
- Comparable sensitivity to Coomassie stain
- Better reproducibility and quantitation compared to staining procedures
- Use of the same gel for downstream western blotting, standard staining methods, and mass spectrometry analysis

## Fastest Separations and Imaging Using Extended Shelf Life Laemmli PAGE

### Introduction

SDS-PAGE is a widely used tool for analyzing protein mixtures. The Laemmli system is regarded as the gold standard for SDS-PAGE techniques, due to its ability to cleanly resolve complex samples from a wide variety of sources in an array of sample backgrounds. SDS-PAGE followed by Coomassie staining is a standard, widely used method to visualize proteins in the gel. However, it involves time-consuming staining and destaining procedures.

The new Mini-PROTEAN and Criterion TGX (Tris-Glycine eXtended) Stain-Free precast gels for PAGE are based on the long-shelf life TGX formulation and include unique trihalo compounds that allow rapid fluorescent detection of proteins with stain-free enabled imagers (for example, the Gel Doc<sup>™</sup> EZ or ChemiDoc<sup>™</sup> MP systems). The TGX Stain-Free gels retain Laemmli-like separation characteristics using the standard sample and Tris/glycine running buffers. The proteins in the gel can be separated in as little as 15 minutes (Mini-PROTEAN TGX Stain-Free gels) and then visualized using either the

Gel Doc EZ or ChemiDoc MP imagers in as little as 2.5 minutes. The trihalo compounds react with tryptophan residues in a UV-induced reaction to produce fluorescence, which is easily detected by stain-free enabled imagers within gels or after transfer onto PVDF membranes. Activation of the trihalo compounds in the gels adds a 58 Da moiety to available tryptophan residues and is required for protein visualization. The sensitivity of the TGX Stain-Free system is comparable to staining with Coomassie Brilliant Blue for proteins with a tryptophan content of at least 1.5%; sensitivity superior to Coomassie staining is possible for proteins with a tryptophan content greater than 3%.

Mini-PROTEAN TGX Stain-Free precast gels are currently available in 7.5%, 10%, 12%, and Any kD<sup>™</sup> formats. The Any kD configuration offers optimal resolution of proteins in the 20–100 kD molecular weight range. Criterion TGX Stain-Free precast gels are available in 7.5%, 10%, 12%, 18%, 4–15%, 4–20%, 8–16%, 10–20%, and Any kD formats. Migration charts for all gel types can be found in Figure 1.

**BIO-RAD**

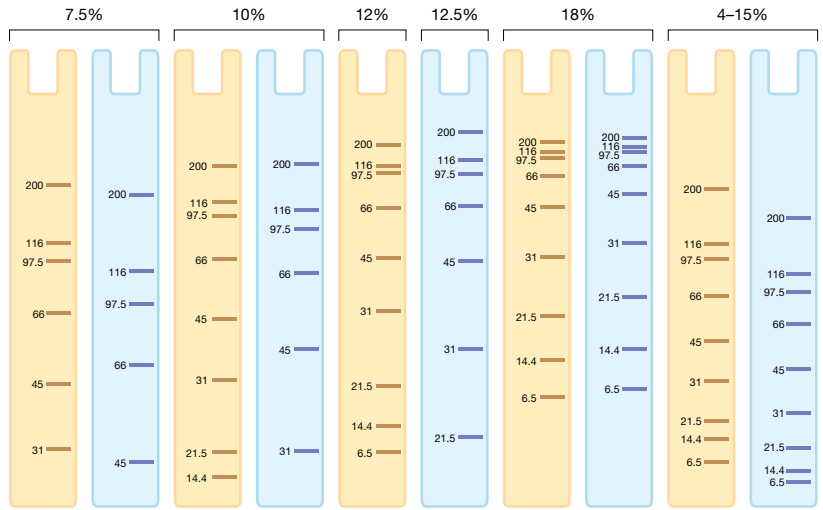


Fig. 1A. Criterion TGX Stain-Free (■) versus Criterion Tris-HCl (■) gel migration charts. Broad range, unstained standards.

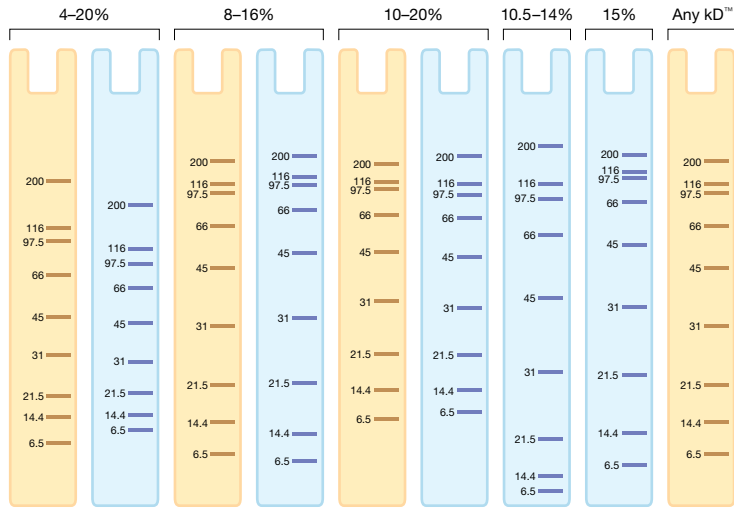
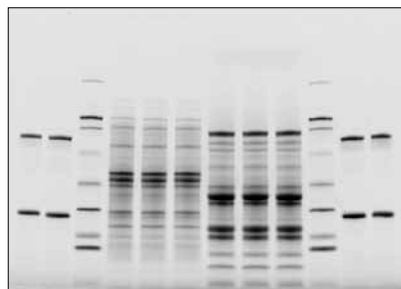


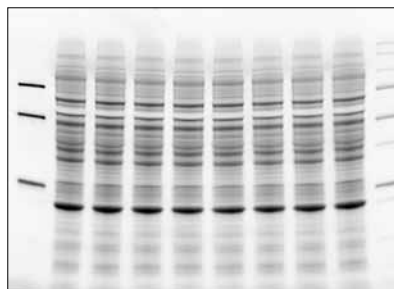
Fig. 1B. Mini-PROTEAN TGX Stain-Free (■) versus Ready Gel® precast gels (■). Broad range, unstained standards.

### Fast and Reproducible Results

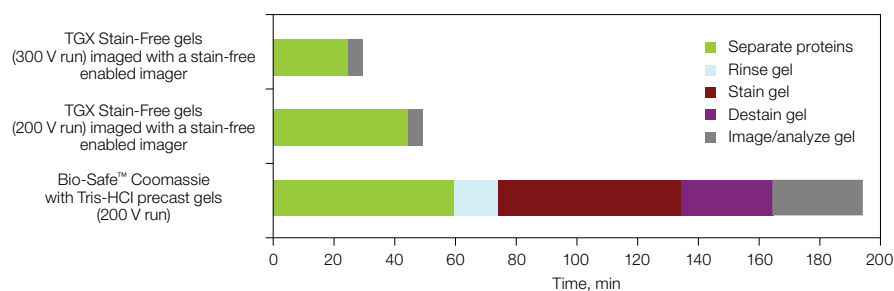
Using TGX Stain-Free precast gels with either the Gel Doc EZ or ChemiDoc MP imagers allows faster separation of proteins, gel imaging, and complete data analysis in as little as 20 minutes. The TGX Stain-Free gels allow faster protein separations at higher voltages without sacrificing band sharpness and resolution (Figures 2 and 3).



**Fig. 2A. Protein separation at higher voltages.** 4–15% Criterion TGX Stain-Free precast gel was run at a constant voltage of 300 V.



**Fig. 2B. Any kD Mini-PROTEAN TGX Stain-Free precast gel was run at a constant voltage of 300 V.**

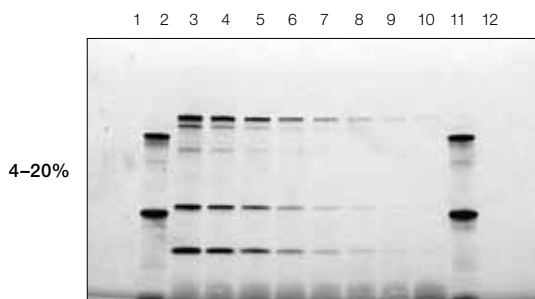


**Fig. 3. Comparison of stain-free technology and Bio-Safe Coomassie staining workflows.** TGX Stain-Free precast gels have shorter run times than the traditional Tris-HCl gels. After electrophoresis, TGX Stain-Free gels take 2.5–5 min to generate results, while Coomassie staining takes at least 2 hr to generate the same level of sensitivity (the graph does not include times for changing solutions).

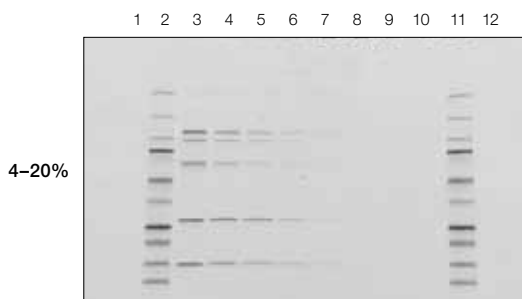
### High Sensitivity

The sensitivity of stain-free technology is equal to or better than that of Coomassie stain. A comparison was done between gels run with broad-range standards and a serial dilution of a sample containing proteins with varying levels of tryptophan content and either imaged on the Gel Doc EZ imager or stained with Bio-Safe Coomassie stain. The gels imaged with the Gel Doc EZ imager showed sensitivity equal to that of Coomassie-stained gels and for some protein bands showed a greater level of sensitivity (Figure 4).

#### A. Gel imaged using Gel Doc EZ imager



#### B. Post-stain with Bio-Safe Coomassie stain

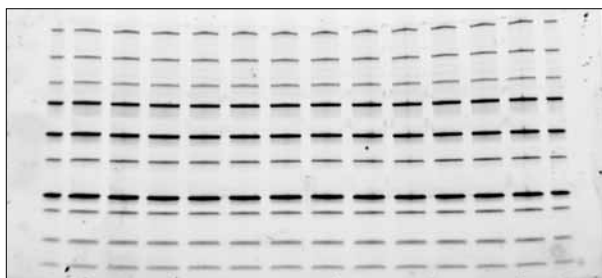


**Fig. 4. Sensitivity comparison of gel images using stain-free technology and Bio-Safe Coomassie stain.** Criterion TGX Stain-Free 4–20% gels were loaded with 6.6  $\mu$ l of Precision Plus Protein™ Dual Color standards in lanes 2 and 11. Lanes 1, 12, and reference lanes are empty. Lanes 3–10 contained 6.6  $\mu$ l of samples made from a serial dilution of a protein mixture containing equal amounts of five different proteins. Bands are numbered from top to bottom of gel, percent tryptophan content for each protein is indicated in parenthesis as % Trp. Band 1,  $\beta$ -galactosidase (3.8% Trp); band 2, phosphorylase B (1.4% Trp); band 3, BSA (0.3% Trp); band 4, carbonic anhydrase (2.3% Trp); band 5, lysozyme (3.4% Trp). The dilution ranged from 338 ng/band to 2.6 ng/band. The gels were imaged with the Gel Doc EZ imager (A), and then stained with Bio-Safe Coomassie stain (B). Note: Some of the bands of the prestained protein standards are undetected by the Gel Doc EZ imager due to interference from the dye. It is recommended to use unstained protein standards with stain-free technology.

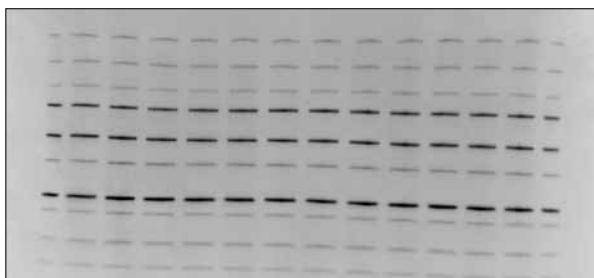
### Greater Transfer Efficiency

TGX Stain-Free precast gels provide fast and excellent transfer efficiency using either wet/tank or semi-dry systems. Protein transfer can be quickly and easily assessed using stain-free enabled imagers. Protein samples retain their fluorescence after the gels are visualized and can be viewed on PVDF membranes after transfer. Stain-free technology provides an excellent tool to monitor both protein separations and transfer quality (Figure 5).

**A. Gel imaged with Gel Doc EZ imager**



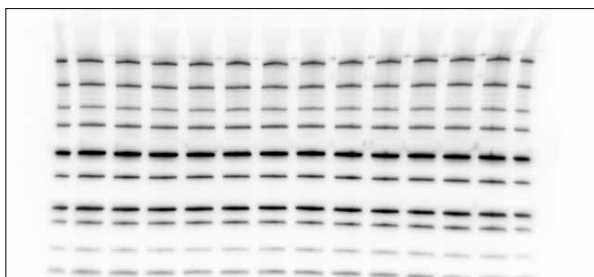
**B. Blot imaged with Gel Doc EZ imager**



**C. Gel imaged after protein transfer**



**D. Chemiluminescent detection of proteins on the blot**



**Fig. 5. Superior resolution of a variety of samples.** Precision Plus Protein Unstained standards were loaded in a volume of 5  $\mu$ l in each lane of 4–20% Criterion TGX Stain-Free gels. The gels were run in Criterion cell at 200 V for 45 min. **A**, protein bands were visualized using the Gel Doc EZ imager; **B**, the separated proteins were then transferred onto an Immun-Blot<sup>®</sup> PVDF membrane using Towbin buffer (with 20% methanol) in a Criterion blotter at 100 V for 1 hr. The transferred proteins on the membrane were also visualized using the Gel Doc EZ imager to confirm transfer efficiency; **C**, the gel was checked for non-transferred proteins; **D**, the blot was probed against a 1:20,000 dilution of StrepTactin-HRP conjugate, developed with Immuno-Star<sup>™</sup> Western C<sup>™</sup> substrate, and visualized on a ChemiDoc XRS system.

## Specifications

### Criterion TGX Stain-Free Gels

Gel dimensions (Width × Length × Thickness)	13.3 × 8.7 × 0.1 cm
Cassette dimensions (Width × Length × Thickness)	15.0 × 10.6 × 0.53 cm
Cassette material	Styrene copolymer
Comb material	Polycarbonate
Gel storage conditions	Store flat at 4°C; do not freeze
Shelf life at 4°C*	12 months
Recommended sample buffer (dilute 1:1 with sample)	Laemmli sample buffer: 62.5 mM Tris-HCl, pH 6.8, 2% SDS, 25% glycerol, 0.01% bromophenol blue (catalog #161-0737)
Recommended running buffer	Tris/Glycine/SDS running buffer: 25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3 (catalog #161-0732)
Run times at:	200 V 300 V
	42–50 min 20–26 min

### Mini-PROTEAN TGX Stain-Free Gels







Gel dimensions (W × L × thickness)	8.6 × 6.7 × 0.1 cm
Cassette dimensions (W × L × thickness)	10.0 × 8.0 × 0.46 cm
Cassette material	Styrene copolymer
Comb material	Polycarbonate
Gel storage conditions	Store flat at 2–8°C; do not freeze
Shelf life at 4°C*	12 months
Recommended sample buffer (Laemmli, dilute 1:1 with sample)	62.5 mM Tris-HCl, pH 6.8, 2% SDS, 25% glycerol, 0.01% bromophenol blue
Recommended running buffer (Tris/Glycine/SDS)	25 mM Tris, 192 mM Glycine, 0.1% SDS, pH 8.3

\* From date of manufacture.






## Related Literature

1658100	Mini-PROTEAN Precast Gels Instruction Manual and Application Guide
4110001	Instruction Manual, Criterion Gel Application Guide
5981	Criterion Tris-HCl to Criterion TGX and TGX Stain-Free Precast Gels Catalog Number Conversion Chart
5932	Ready Gel to Mini-PROTEAN TGX and TGX Stain-Free Precast Gels Catalog Number Conversion Chart
5934	NuPAGE Precast Gels to MINI-PROTEAN TGX and TGX Stain-Free Precast Gels Catalog Number Conversion Chart
5976	Gel Doc EZ Imaging System Brochure
6133	ChemiDoc MP Imaging System Brochure
6032	Life Technologies Bis-Tris and Tris-Glycine to Criterion TGX/TGX Stain-Free Precast Gels Conversion Chart
6008	Comparison of the Criterion TGX Stain-Free Precast Gel System and Standard Coomassie Staining Procedures for Running and Imaging Protein Gels

## Ordering Information

Description	 10-Well 30 µl	 10-Well 50 µl	 15-Well 15 µl	 IPG/prep 450 µl	 12-Well 20 µl	 8+1-Well 30 µl
<b>Mini-PROTEAN TGX Stain-Free Precast Gels</b>						
7.5%	456-8023	456-8024	456-8026	456-8021	456-8025	456-8029
10%	456-8033	456-8034	456-8036	456-8031	456-8035	456-8039
12%	456-8043	456-8044	456-8046	456-8041	456-8045	456-8049
Any kD	456-8123	456-8124	456-8126	456-8121	456-8125	456-8129

All formats are available as both ten packs (catalog numbers listed) and two packs. To order as a two pack, add an "S" to the end of the catalog number for the corresponding ten pack.

Description	 12+2*-Well 45 µl	 18-Well 30 µl	 26-Well 15 µl	 Prep+2*-Well 800 µl	 IPG+1*-Well 11 cm IPG Strip
<b>Criterion TGX Stain-Free Precast Gels**</b>					
7.5% Gel	567-8023	567-8024	567-8025	—	—
10% Gel	567-8033	567-8034	567-8035	—	—
12% Gel	567-8043	567-8044	567-8045	—	—
18% Gel	567-8073	567-8074	567-8075	567-8072	567-8071
4–15% Gel	567-8083	567-8084	567-8085	567-8082	567-8081
4–20% Gel	567-8093	567-8094	567-8095	567-8092	567-8091
8–16% Gel	567-8103	567-8104	567-8105	567-8102	567-8101
10–20% Gel	567-8113	567-8114	567-8115	567-8112	567-8111
Any kD Gel	567-8123	567-8124	567-8125	567-8122	567-8121

\* Reference well accommodates 15 µl of markers/standards.

\*\* Criterion TGX Stain-Free gels are sold as a single gel.

## Ordering Information (contd.)

Catalog # Description

### Electrophoresis Cells

#### Mini-PROTEAN Tetra Cell

- 165-8004 **Mini-PROTEAN Tetra Cell for Mini Precast Gels**, 4-gel system includes electrode assembly, clamping frame, companion module, tank, lid with power cables, mini cell buffer dam
- 165-8005\* **Mini-PROTEAN Tetra Cell for Mini Precast Gels**, 2-gel system includes electrode assembly, clamping frame, tank, lid with power cables, mini cell buffer dam
- 165-8030 **Mini-PROTEAN Tetra Cell for Mini Precast Gels and Mini Trans-Blot Module**, includes 165-8004 and 170-3935
- 165-8034 **Mini-PROTEAN Tetra Cell for Mini Precast Gels, Mini Trans-Blot Module, and PowerPac™ Basic Power Supply**, includes 165-8004, 170-3935, and 164-5050
- 165-8036 **Mini-PROTEAN Tetra Cell for Mini Precast Gels, Mini Trans-Blot Module, and PowerPac HC Power Supply**, includes 165-8004, 170-3935, and 164-5052

#### Mini-PROTEAN® 3 Dodeca™ Cell

- 165-4100 **Mini-PROTEAN 3 Dodeca Cell**, includes electrophoresis tank with built-in cooling coil, lid with power cables, 6 electrophoresis clamping frames, 2 buffer dams, drain line, 2 gel releasers

#### Criterion Cell

- 165-6001 **Criterion Cell**, includes electrophoresis buffer tank, lid with power cables, 3 sample loading guides (12+2-well, 18-well, 26-well)

#### Criterion™ Dodeca™ Cell

- 165-4130 **Criterion Dodeca Cell**, includes electrophoresis buffer tank with built-in cooling coil, lid with power cables

### Buffers and Reagents

#### Sample Buffers

- 161-0737 **Laemmli Sample Buffer**, 30 ml
- 161-0738 **Native Sample Buffer**, 30 ml
- 161-0610 **Dithiothreitol (DTT)**, 1 g
- 161-0611 **Dithiothreitol (DTT)**, 5 g
- 161-0710 **2-Mercaptoethanol**, 25 ml

#### Running Buffers

- 161-0732 **10x Tris/Glycine/SDS**, 1 L
- 161-0772 **10x Tris/Glycine/SDS**, 5 L cube
- 161-0771 **10x Tris/Glycine**, 5 L cube
- 161-0416 **SDS Solution**, 10% (w/v), 250 ml
- 161-0418 **SDS Solution**, 20% (w/v), 1 L

### Imaging Systems

- 170-8270 **Gel Doc EZ System**
- 170-8271 **UV Sample Tray**, Pkg of 1

### Stains

#### Bio-Safe Coomassie Stain

- 161-0786 **Bio-Safe Coomassie Stain**, 1 L
- 161-0787 **Bio-Safe Coomassie Stain**, 5 L

Catalog # Description

### Silver Stain

- 161-0449 **Silver Stain Plus™ Kit**, includes fixative enhancer concentrate, silver complex solution, reduction moderator solution, image development reagent, development accelerator reagent, stains 13 full size or 40 mini gels
- 161-0443 **Silver Stain Kit**, includes oxidizer concentrate, silver reagent concentrate, silver stain developer, stains 20 full size or 48 mini gels

### Flamingo™ Stain

- 161-0490 **Flamingo Fluorescent Gel Stain**, 10× solution, 20 ml
- 161-0491 **Flamingo Fluorescent Gel Stain**, 10× solution, 100 ml
- 161-0492 **Flamingo Fluorescent Gel Stain**, 10× solution, 500 ml

### SYPRO Ruby Stain

- 170-3126 **SYPRO Ruby Protein Gel Stain**, 1× solution, 200 ml
- 170-3125 **SYPRO Ruby Protein Gel Stain**, 1× solution, 1 L
- 170-3138 **SYPRO Ruby Protein Gel Stain**, 1× solution, 5 L

### Protein Standards

#### Precision Plus Protein Standards

- 161-0363 **Precision Plus Protein Unstained Standards**, 1 ml, 100 applications
- 161-0377 **Precision Plus Protein Dual Xtra Standards**, 500 µl, 50 applications
- 161-0373 **Precision Plus Protein All Blue Standards**, 500 µl, 50 applications
- 161-0374 **Precision Plus Protein Dual Color Standards**, 500 µl, 50 applications
- 161-0375 **Precision Plus Protein™ Kaleidoscope™ Standards**, 500 µl, 50 applications
- 161-0376 **Precision Plus Protein™ WesternC™ Standards**, 250 µl, 50 applications
- 161-0385\* **Precision Plus Protein WesternC Pack**, 50 applications

#### Natural Unstained Standards

- 161-0303 **SDS-PAGE Standards**, high range, 200 µl
- 161-0304 **SDS-PAGE Standards**, low range, 200 µl
- 161-0317 **SDS-PAGE Standards**, broad range, 200 µl

\* Each pack includes 250 µl of Precision Plus Protein WesternC standards and 125 µl of StrepTactin-HRP conjugate.

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Precision Plus Protein standards are sold under license from Life Technologies Corporation, Carlsbad, CA, for use only by the buyer of the product. The buyer is not authorized to sell or resell this product or its components.

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