

# 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 UVinduced 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%, 4–15%, 4–20%, 8–16%, and Any kD<sup>™</sup> formats. The Any kD configuration offers optimal resolution of proteins in the 10–100 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.



7.5%		10	%	12%	12.5%	18%		4–15%	
200	200 —	200 — 116 — 97.5 —	200 — 116 — 97.5 —	200 — 116 97.5 — 66 —	200 — 116 — 97.5 — 66 —	200 116 97.5 66 45	200 116 97 66 45	200-	200
97.5	116	66 —	66 —	45	45 —	31 —	31	97.5	116
66	97.5 —	45 —		31 —		21.5	21.5	66 —	97.5
45 —	66 —	31 —	45 —	21.5 —	31 —	14.4 <del></del> 6.5 <del></del>	14.4 <b>—</b> 6.5 <b>—</b>	45 <b>—</b> 31 <b>—</b>	45
31 —	45 —	21.5 <b>—</b> 14.4 <b>—</b>	31 —	14.4 <b>—</b> 6.5 <b>—</b>	21.5 —			21.5 — 14.4 — 6.5 —	31 21.5 14.4 6.5
4-2	0%	8–11	6%		20%	10.5–14%	15%	Any kD™	
200 — 116 — 97.5 — 66 — 45 — 31 — 21.5 — 14.4 — 6.5 —	200 — 116 — 97.5 — 45 — 31 — 21.5 — 14.4 — 6.5 —	200 — 9 <sup>1</sup> /5 — 45 — 31 — 21.5 — 14.4 — 6.5 —	200 97.5 66 45 31 21.5 14.4 55	200 16 97.5 45 31 21.5 14.4 6.5	200 — 116 — 97.5 — 66 — 45 — 31 — 21.5 — 14.4 — 6.5 —	200 — 116 — 97.5 — 66 — 45 — 31 — 21.5 —	200 116 97.5 66 45 31 21.5 14.4	200 — 116 — 97.5 — 66 — 45 — 31 — 21.5 — 14.4 — 65 —	

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<sup>®</sup> 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. 3. Comparison of stain-free technology and Bio-Safe Coomassie staining workflows. TGX Stain-Free Precast Gels have shorter run times than the traditional Trie-HCI aels. After electrophonesis, TGX Stain-Free Gels take 2.5–5 min to generate results, while Coomassie staining

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).



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



Fig. 4. Sensitivity comparison of gel images using stain-free technology and Bio-Safe Coomassie Stain. 4–20% Criterion TGX Stain-Free Gels were loaded with 6.6  $\mu$ l of Precision Plus Protein<sup>™</sup> 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).





#### B. Blot imaged with Gel Doc EZ Imager



D. Chemiluminescent detection of proteins on the blot

C. Gel imaged after protein transfer



Fig. 5. Superior resolution of a variety of samples. Precision Plus Protein Unstained Standards were loaded in a volume of 5 µl in each lane of 4–20% Criterion TGX Stain-Free Gels. The gels were run in a 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 Immun-Star<sup>™</sup> WesternC<sup>™</sup> Substrate, and visualized using the ChemiDoc<sup>™</sup> XRS System.

## **Specifications**

13.3 × 8.7 × 0.1 cm 15.0 × 10.6 × 0.53 cm				
Polycarbonate				
Store flat at 4°C; do not freeze				
12 months				
Laemmli Sample Buffer: 62.5 mM Tris-HCl, pH 6.8, 2% SDS, 25% glycerol, 0.01% bromophenol blue (catalog #161-0737)				
Tris/glycine/SDS running buffer: 25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3 (catalog #161-0732)				
42–50 min				
20–26 min				
$8.6 \times 6.7 \times 0.1$ cm				
$10.0 \times 8.0 \times 0.46$ cm				
Styrene copolymer				
Polycarbonate				
Store flat at 2–8°C; do not freeze				
12 months				
62.5 mM Tris-HCl, pH 6.8, 2% SDS, 25% glycerol, 0.01% bromophenol blue				
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**

<b>-</b>		·	······			
	10-Well	10-Well	15-Well	IPG/prep	12-Well	8+1-Well
Description	30 µl	50 µl	15 µl	250 µl	20 µl	30 µl
Mini-PROTEAN TGX Stain-Free	Precast Gels					
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
4–15%	456-8083	456-8084	456-8086	456-8081	456-8085	456-8089
4–20%	456-8093	456-8094	456-8096	456-8091	456-8095	456-8099
8–16%	456-8103	456-8104	456-8106	456-8101	456-8105	456-8109
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 ul	18-Well	26-Well	Prep+2*-Well	IPG+1*-Well
Criterion TGX Stain-Free P	recast Gels**	ου μι	10 µ	700 μι	
7.5%	567-8023	567-8024	567-8025	_	_
10%	567-8033	567-8034	567-8035	_	_
12%	567-8043	567-8044	567-8045	_	_
18%	567-8073	567-8074	567-8075	567-8072	567-8071
4–15%	567-8083	567-8084	567-8085	567-8082	567-8081
4–20%	567-8093	567-8094	567-8095	567-8092	567-8091
8–16%	567-8103	567-8104	567-8105	567-8102	567-8101
10–20%	567-8113	567-8114	567-8115	567-8112	567-8111
Any kD	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.

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<b>Ordering In</b>	formation (contd.)	
Catalog #	Description	Catalog #
Electrophores Mini-PROTEAN	is Cells N Tetra Cell	<b>Silver Stain</b> 161-0449
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	161-0443
165-8030	Mini-PROTEAN Tetra Cell for Mini Precast Gels and Mini Trans-Blot Module, includes 165-8004 and 170-3935	<b>Flamingo</b> ™ \$ 161-0490
165-8034	Mini-PROTEAN Tetra Cell for Mini Precast Gels, Mini Trans-Blot Module, and PowerPac <sup>™</sup> Basic Power Supply, includes 165-8004, 170-3935 and 164-5050	161-0491 161-0492 SYPRO Rub
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	170-3126 170-3125 170-3138
Mini-PROTEA	N <sup>®</sup> 3 Dodeca <sup>™</sup> Cell	Protein Sta
165-4100	Mini-PROTEAN 3 Dodeca Cell, includes electrophoresis tank with built-in cooling coil, lid with power cables, 6 electrophoresis clamping frames,	161-0363
	2 buffer dams, drain line, 2 gel releasers	161-0377
Criterion Cell 165-6001	Criterion Cell, includes electrophoresis buffer tank,	161-0373
	(12+2-well, 18-well, 26-well)	161-0374
Criterion <sup>™</sup> Doc	leca™ Cell	161-0375
165-4130	Criterion Dodeca Cell, includes electrophoresis buffer tank with built-in cooling coil, lid with power cables	161-0376
Buffers and Re	eagents	161-0385*
Sample Buffer	S	
161-0737 161-0747 161-0738 161-0610	Laemmli Sample Buffer, 30 ml 4x Laemmli Sample Buffer, 10 ml Native Sample Buffer, 30 ml Dithiothreitol (DTT), 1 g	Natural Uns 161-0303 161-0304 161-0317
161-0710	2-Mercaptoethanol, 25 ml	* Each pack ir Standards a
161-0732	10x Tris/Glycine/SDS. 1	Bio-Rad Labo
161-0772	10x Tris/Glycine/SDS, 5 L cube	to sell SYPRC
161-0771	10x Tris/Glycine, 5 L cube	5,616,502.
161-0416 161-0418	SDS Solution, 10% (w/v), 250 ml SDS Solution, 20% (w/v), 1 L ms	Precision Plus Technologies the product. 7
170-8270	Gel Doc EZ System	or its compon
170-8271	UV Sample Tray, pkg of 1	StrepTactin is
Stains Bio-Safe Coon	nassie Stain	Bio-Rad Labo to sell these p

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

concentrate, silver complex solution, reduction moderator solution, image development reagent, development accelerator reagent, stains 13 full size or 40 mini aels 61-0443 Silver Stain Kit, includes oxidizer concentrate, silver reagent concentrate, silver stain developer, stains 20 full size or 48 mini gels Flamingo<sup>™</sup> Stain 61-0490 Flamingo Fluorescent Gel Stain, 10× solution, 20 ml 61-0491 Flamingo Fluorescent Gel Stain, 10× solution, 100 ml 61-0492 Flamingo Fluorescent Gel Stain, 10× solution, 500 ml SYPRO Ruby Stain 70-3126 SYPRO Ruby Protein Gel Stain, 1× solution, 200 ml 70-3125 SYPRO Ruby Protein Gel Stain, 1× solution, 1 L 70-3138 SYPRO Ruby Protein Gel Stain, 1× solution, 5 L Protein Standards Precision Plus Protein Standards Precision Plus Protein Unstained Standards, 161-0363 1 ml. 100 applications 61-0377 Precision Plus Protein Dual Xtra Standards, 500 ul 50 applications 61-0373 Precision Plus Protein All Blue Standards, 500 µl, 50 applications 61-0374 Precision Plus Protein Dual Color Standards, 500 µl, 50 applications 61-0375 Precision Plus Protein<sup>™</sup> Kaleidoscope<sup>™</sup> Standards, 500 µl, 50 applications 61-0376 Precision Plus Protein<sup>™</sup> WesternC<sup>™</sup> Standards, 250 µl, 50 applications 61-0385\* Precision Plus Protein WesternC Pack, 50 applications Natural Unstained Standards 61-0303 SDS-PAGE Standards, high range, 200 µl 161-0304 SDS-PAGE Standards, low range, 200 µl 61-0317 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.

Description

Silver Stain Plus<sup>™</sup> Kit. includes fixative enhancer

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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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