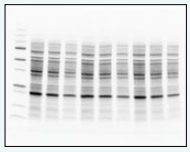
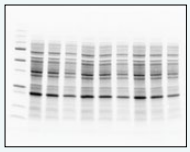
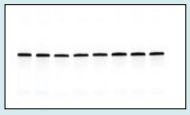
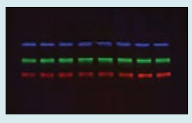
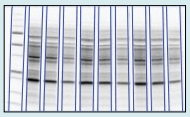
Bio-Rad **Stain-Free** Western Blotting

Faster Results, Better Data

Compared to conventional methods, this improved workflow, incorporating Stain-Free gel technology, saves time and increases the accuracy and reliability of your western blot results.

Workflow	Benefit
Separate Proteins 1 	**Run four mini or two midi gels in as little as 15 min** ▪ Speed with flexibility: TGX Stain-Free Gel chemistry available in precast and handcast formats
Visualize Protein Separation 2   Stain-Free image of **pretransferred gel**	**Visualize separation for all lanes in 1 min** ▪ Coomassie-like performance with no background variability and no staining/destaining
Transfer 3 	**Efficient and uniform protein transfer in 3 min** ▪ Throughput: transfer 4 mini or 2 midi gels at once ▪ Convenience: choose from ready-to-use transfer packs (nitrocellulose or polyvinylidene difluoride [PVDF]) or ready-to-assemble kits (nitrocellulose, PVDF, or low fluorescence PVDF)
Assess Transfer Efficiency 4   Stain-Free image of **blot**	**Quickly check transfer efficiency** ▪ Verify quality of transfer for all lanes in 2 min
Antibody Incubation and Blot Detection ~5 hr	
Normalize Total Protein and Analyze Western Blot Data 5   Target protein detection with chemiluminescence  Target protein detection with fluorescence  Stain-Free blot (from step 4) for normalization	**Easy multiplexing** ▪ Use western blot–validated, highly specific PrecisionAb Primary Antibodies and our StarBright Blue Fluorescent Secondary Antibodies to detect multiple targets on the same blot **Use Stain-Free blot image as total protein loading control** ▪ No need to strip and reprobe or cut the blot ▪ Use all the proteins in the sample to normalize rather than depending on a single housekeeping protein ▪ Reliable and accurate quantitation

BIO-RAD

FAQs

How does Stain-Free technology work?

The embedded compound in Stain-Free gels is activated and imaged, postelectrophoresis, by a Bio-Rad imager to provide a fluorescent signal with sensitivity equivalent to Coomassie staining. The Stain-Free signal transfers with the proteins during blotting, which enables the assessment of transfer efficiency and provides total protein signal for use in normalization.

How do total protein stains compare to normalizing with a housekeeping protein?

Advantages of total protein stains vs. housekeeping normalization include:

1. Housekeeping protein (HKP) expression may change depending on experimental conditions and sample type. Total protein is more stable and less prone to change with experimental conditions.
2. Total protein normalization using Stain-Free technology eliminates the need to strip and reprobe for housekeeping proteins. Stripping and reprobing for HKP is a very time-consuming process, adding hours to detection, whereas Stain-Free total protein detection can be done in minutes.
3. Compared to housekeeping proteins, total protein stains exhibit superior linearity and reproducibility.

How does Stain-Free fluorescence compare to other total protein stains for blots?

Stain-Free technology provides higher detection sensitivity and a wider linear dynamic range than available total protein stains. Stain-Free technology eliminates staining and destaining manipulation, ensuring proteins remain intact from gel to membrane. Other total protein stains, including Ponceau S and SYPRO Ruby, can introduce errors in the staining/destaining procedure.

What resources are available?

Tips on normalization with Stain-Free technology are available at **bio-rad.com**.

Bulletin 6434	Western Blot Normalization Using Image Lab Software
Bulletin 6390	General Stain-Free Western Blotting Protocol
Bulletin 6360	A Method for Greater Reliability in Western Blot Loading Controls: Stain-Free Total Protein Quantitation
Bulletin 6351	Selected Publications List: Total Protein Normalization in Western Blotting Using Stain-Free Technology

Tips for good quantitative western blotting are available at **bio-rad.com**.

Bulletin 2895	Protein Blotting Guide

bio-rad.com/tech/westernblotdoctor

Ordering Information

Catalog # Description

Protein Standards

1610373 **Precision Plus Protein All Blue Standards**
1610363 **Precision Plus Protein Unstained Standards**

Buffers

1610732 **10x Tris/Glycine/SDS Buffer**
1610747 **4x Laemmli Sample Buffer**

Electrophoresis Cell

1656001 **Criterion Cell**, includes electrophoresis buffer tank, lid with power cables, 3 sample loading guides
1658004 **Mini-PROTEAN Tetra Cell for Mini Precast Gels**, 4-gel vertical electrophoresis system, includes electrode assembly, companion running module, tank, lid with power cables, mini cell buffer dam

Blotting System

17001917 **Trans-Blot Turbo Transfer Starter System**, mini, PVDF
17001919 **Trans-Blot Turbo Transfer Starter System**, midi, PVDF
17001918 **Trans-Blot Turbo Transfer Starter System**, mini, nitrocellulose
17001915 **Trans-Blot Turbo Transfer Starter System**, midi, nitrocellulose
1704156 **Trans-Blot Turbo Transfer Pack**, mini, PVDF, pkg of 10
1704157 **Trans-Blot Turbo Transfer Pack**, midi, PVDF, pkg of 10
1704158 **Trans-Blot Turbo Transfer Pack**, mini, nitrocellulose, pkg of 10
1704159 **Trans-Blot Turbo Transfer Pack**, midi, nitrocellulose, pkg of 10
1704272 **Trans-Blot Turbo RTA Transfer Kit**, mini, PVDF
1704273 **Trans-Blot Turbo RTA Transfer Kit**, midi, PVDF
1704274 **Trans-Blot Turbo RTA Transfer Kit**, mini, low fluorescence PVDF
1704275 **Trans-Blot Turbo RTA Transfer Kit**, midi, low fluorescence PVDF
1704270 **Trans-Blot Turbo RTA Transfer Kit**, mini, nitrocellulose
1704271 **Trans-Blot Turbo RTA Transfer Kit**, midi, nitrocellulose

Imaging Systems

12009077 **GelDoc Go Gel Imaging System**
12003153 **ChemiDoc Imaging System**
12003154 **ChemiDoc MP Imaging System**
1708265 **ChemiDoc XRS+ System with Image Lab Software**

Detection Reagents

1705060 **Clarity Western ECL Substrate**, 200 ml
1705061 **Clarity Western ECL Substrate**, 500 ml
1705062 **Clarity Max Western ECL Substrate**, 100 ml
12004158 **StarBright Blue 700 Goat Anti-Mouse IgG**, 400 µl
12004161 **StarBright Blue 700 Goat Anti-Rabbit IgG**, 400 µl
12005866 **StarBright Blue 520 Goat Anti-Mouse IgG**, 400 µl
12005869 **StarBright Blue 520 Goat Anti-Rabbit IgG**, 400 µl
12004163 **hFAB Rhodamine Anti-Actin Primary Antibody**, 200 µl
12004165 **hFAB Rhodamine Anti-Tubulin Primary Antibody**, 200 µl
12004167 **hFAB Rhodamine Anti-GAPDH Primary Antibody**, 200 µl

TGX Stain-Free Precast Gels

Description	10-Well 30 µl	10-Well 50 µl	12-Well 20 µl	15-Well 15 µl	IPG Well 7 cm IPG Strip
Mini-PROTEAN TGX Stain-Free Precast Gels					
7.5% Resolving Gel	4568023	4568024	4568025	4568026	4568021
10% Resolving Gel	4568033	4568034	4568035	4568036	4568031
12% Resolving Gel	4568043	4568044	4568045	4568046	4568041
4–15% Resolving Gel	4568083	4568084	4568085	4568086	4568081
4–20% Resolving Gel	4568093	4568094	4568095	4568096	4568091
8–16% Resolving Gel	4568103	4568104	4568105	4568106	4568101
Any kD Resolving Gel	4568123	4568124	4568125	4568126	4568121

Description	12+2-Well* 45 µl	18-Well 30 µl	26-Well 15 µl	Prep+2-Well* 700 µl	IPG+1-Well* 11 cm IPG Strip
Criterion TGX Stain-Free Precast Gels**					
7.5% Gel	5678023	5678024	5678025	—	—
10% Gel	5678033	5678034	5678035	—	—
12% Gel	5678043	5678044	5678045	—	—
4–15% Gel	5678083	5678084	5678085	5678082	5678081
4–20% Gel	5678093	5678094	5678095	5678092	5678091
8–16% Linear Gradient	5678103	5678104	5678105	5678102	5678101
Any kD Gel	5678123	5678124	5678125	5678122	5678121

* Reference wells accommodate 15 µl of markers/standards.
** Criterion TGX Stain-Free Gels are sold singly.

Visit **bio-rad.com/StainFreeWestern** for more information.