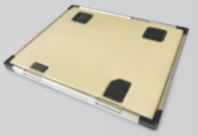
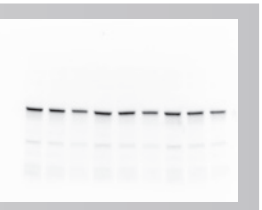
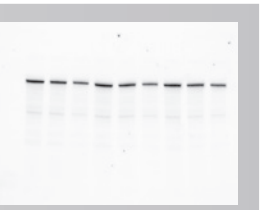
Stain-Free Western Blotting

Faster Results. Better Data.

BIO-RAD

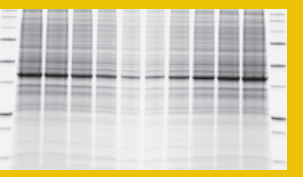
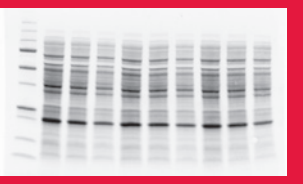
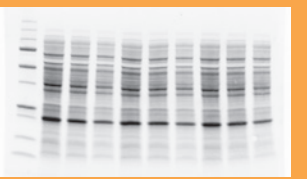
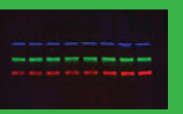
Traditional Workflow

Total Time: 16 hr

Step	Workflow		Time	Data
1	Gel Preparation		>1 hr	—
2	Electrophoresis		~1 hr	—
3	Transfer		1–3 hr	—
4	Antibody Incubation		~5 hr	—
5	Imaging and Analysis		>30 min	 Target proteins
6	Strip and Reprobe Often need reprobing for actin/tubulin as loading control		~5 hr	 Loading control

Stain-Free Western Blotting

Total Time: 5 hr

Step	Workflow		Time	Data
1	**Separate** Proteins		~15 min	 Protein separation at 300 V
2	**Visualize** Separation		1 min	 Stain-Free image of pretransferred gel
3	**Transfer** Proteins		3–7 min	—
4	**Assess** Transfer		2 min	 Stain-Free image of blot
5	**Analyze** Western Blot by Total Protein Normalization		~4.5 hr	 Chemiluminescence detection  Fluorescence detection

Stain-Free Western Blotting

Separate Proteins

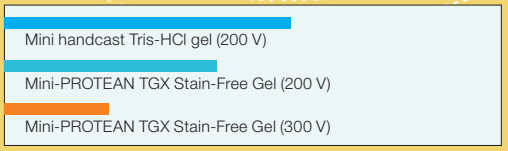
1

Rapidly separate proteins in as little as 15 minutes with Criterion and Mini-PROTEAN TGX Stain-Free Precast Gels.

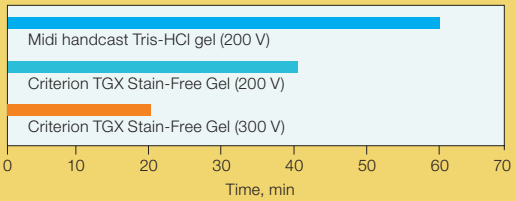
TGX chemistry offers superior protein separation with fast run times, features a one-year shelf life, and uses standard Tris-glycine running buffers.

Stain-Free technology is a sensitive, time-saving alternative to traditional Coomassie staining and is compatible with western blotting. No staining or destaining is required, offering a streamlined workflow.

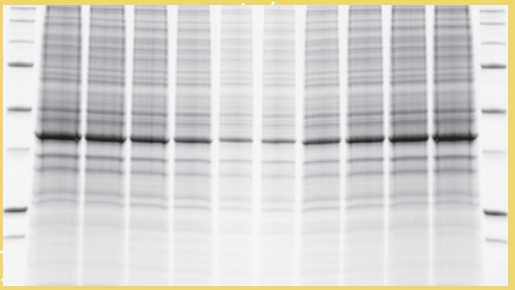
Mini gel typical run times



Midi gel typical run times



Protein separation at 300 V



Protein separation at 200 V

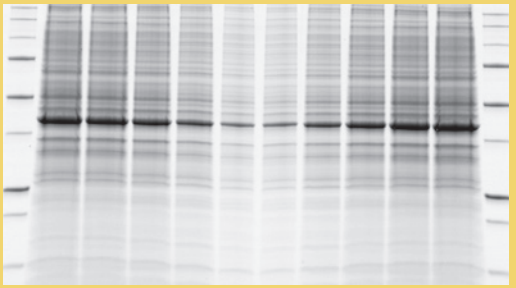
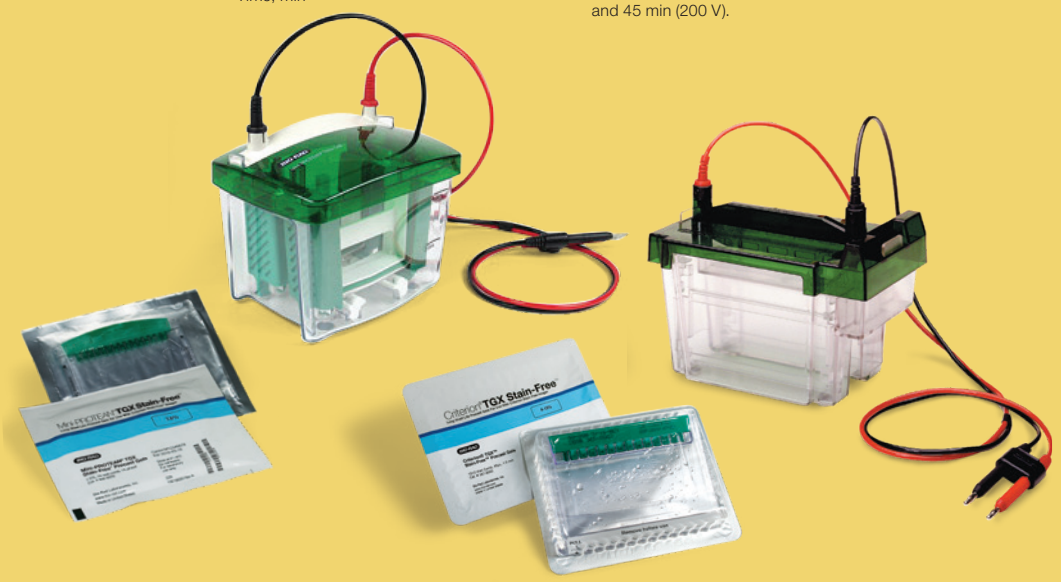


Fig. 1. Protein separation at 300 V or 200 V yields similar results. Any kD Criterion TGX Stain-Free Precast Gels run for 30 min (300 V) and 45 min (200 V).



Faster Results. Better Data.

Visualize Separation

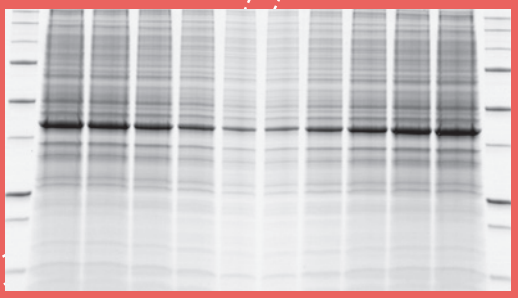
2

Immediately visualize separation with Stain-Free technology and the ChemiDoc MP Imaging System in one easy step.

Advantages:

- Ensure all proteins are separated
- Save time staining proteins (1 min vs. 2 hr for Coomassie staining)
- Run just 1 gel (Coomassie staining requires a separate gel)

Stain-Free gel activation and imaging (1 min)



Coomassie-stained gel (overnight staining)

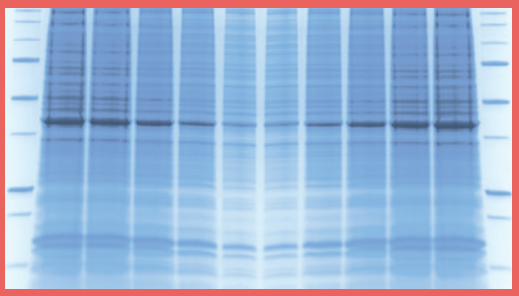
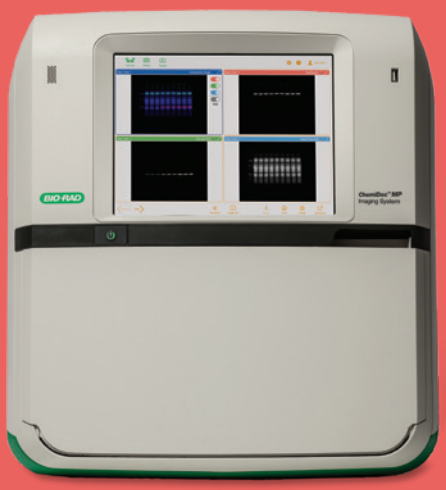


Fig. 2. Results using a Stain-Free gel are similar to those using a Coomassie-stained gel. Any kD Criterion TGX Stain-Free Precast Gel run at 200 V for 45 min. Stain-Free technology is visualized on the ChemiDoc MP Imaging System and compared to a gel stained overnight with a Bio-Safe Coomassie Stain.



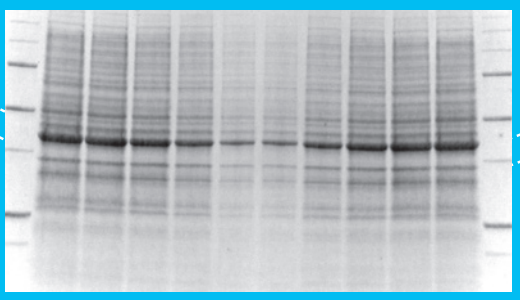
Transfer Proteins

3

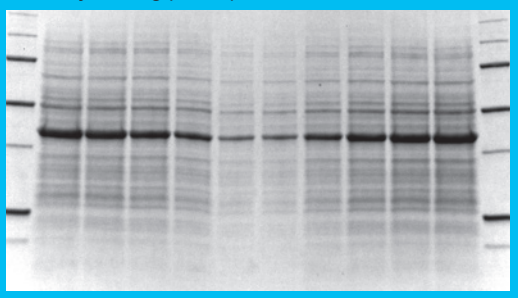
The Trans-Blot Turbo Transfer System enables rapid and efficient transfer of proteins across a wide range of molecular weights.

The Trans-Blot Turbo System offers rapid protein transfer efficiency — achieved in as little as 3 minutes — compared to tank blotting.

Trans-Blot Turbo System (7 min)



Semi-dry blotting (30 min)



Tank blotting (60 min)

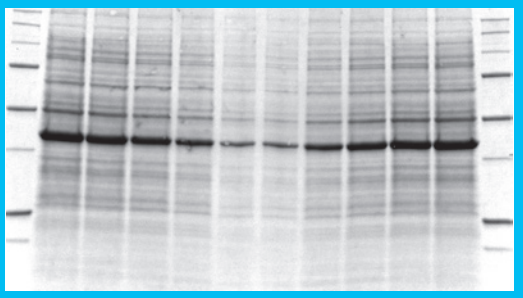


Fig. 3. Transfer efficiencies are comparable between the Trans-Blot Turbo System and tank blotting. Transfers were performed with the Trans-Blot Turbo System (7 min), the Trans-Blot SD Semi-Dry Transfer Cell (30 min), and by tank transfer (60 min).

Assess Transfer

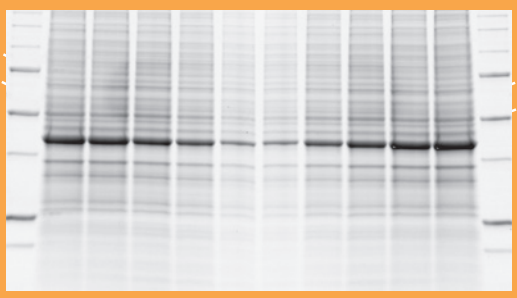
4

Verify high-quality transfer using Stain-Free technology by instantly imaging the membrane on the ChemiDoc MP Imaging System.

Advantages:

- Check transfer efficiency
- Detect air bubbles and uneven transfers
- No need for separate Ponceau S staining

Stain-Free technology



Ponceau S Stain

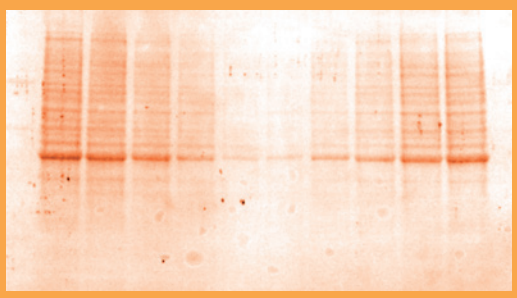
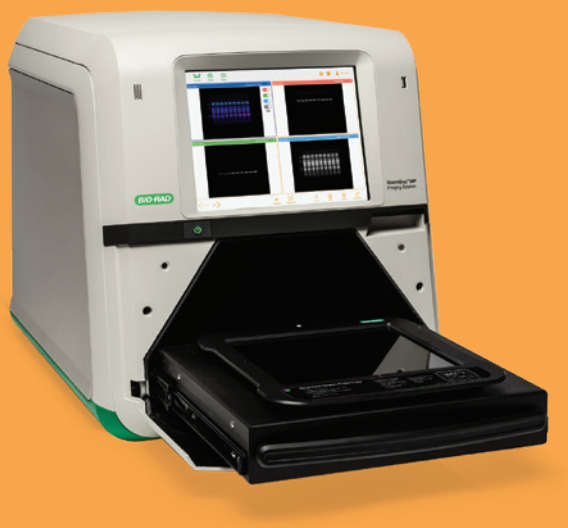


Fig. 4. Stain-Free technology has a higher protein staining efficiency compared to the Ponceau S Stain. Visualization of the previously activated Stain-Free technology for total protein on a blot compared with a membrane stained with the Ponceau S Stain for 1–2 min.



Analyze Western Blot by Total Protein Normalization

5

Perform multiplex or chemiluminescent blot detection and total protein normalization using the ChemiDoc MP Imaging System and Image Lab Software.

The ideal western blot normalization signal:

- Provides 1:1 change in signal with change in total protein loaded
- Is linear over the widest range of total protein loaded
- Does not depend on the expression of a single protein being constant for all experimental treatments

Total protein normalization is **recommended** because it satisfies all three characteristics.

Housekeeping protein (HKP) normalization is **not recommended** because the signal:

- Depends on the expression of a single protein being constant for all experimental treatments
- Tends to be linear only at a low amount of total protein load (up to ~10 µg/lane)

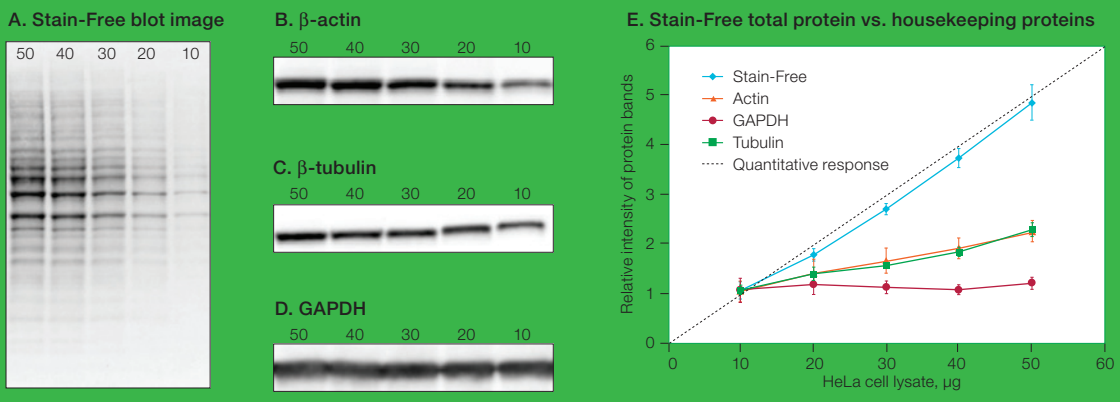


Fig. 5. Total protein detection using Stain-Free technology provides a 1:1 signal relative to protein load over a wide range of total protein load. **A**, Stain-Free total protein signal. **B**, β-actin signal. **C**, β-tubulin signal. **D**, GAPDH signal. **E**, comparison of linearity of total protein normalization vs. housekeeping proteins over a range of protein concentrations.

Ordering Information

Mini-PROTEAN 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
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

Criterion TGX Stain-Free Precast Gels**

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
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% Gel	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.

Catalog #	Description
Imaging Systems	
12003154	**ChemiDoc MP Imaging System**, blot and gel imaging system, UV/visible light imaging, chemiluminescence, 5 fluorescence channels (RGB, far red, near-IR). Includes internal computer, 12" touch screen display, Image Lab Touch Software, blot/UV/Stain-Free sample tray
12003153	**ChemiDoc Imaging System**, blot and gel imaging system, UV/visible light imaging, chemiluminescence, upgradeable for multiplex fluorescence detection. Includes internal computer, 12" touch screen display, Image Lab Touch Software, blot/UV/Stain-Free sample tray
Protein Standards	
1610373	**Precision Plus Protein All Blue Standards**
1610363	**Precision Plus Protein Unstained Standards**
1610385	**Precision Plus Protein WesternC Pack**
Buffers	
1610732	**10x Tris/Glycine/SDS**
1610747	**4x Laemmli Sample Buffer**
Electrophoresis Cells	
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

Catalog #	Description
Power Supplies	
1645050	**PowerPac Basic Power Supply**
1645070	**PowerPac Universal Power Supply**
Blotting Materials	
1704150	**Trans-Blot Turbo Transfer System**, blotting instrument, includes base, 2 cassettes to hold 1–2 midi or up to 4 mini blotting sandwiches, blot roller
1704156	**Trans-Blot Transfer Pack**, mini, PVDF, pkg of 10
1704157	**Trans-Blot Transfer Pack**, midi, PVDF, pkg of 10
1704158	**Trans-Blot Transfer Pack**, mini, nitrocellulose, pkg of 10
1704159	**Trans-Blot Transfer Pack**, midi, nitrocellulose, pkg of 10
1704270	**Trans-Blot Turbo RTA Transfer Kit**, mini, nitrocellulose
1704271	**Trans-Blot Turbo RTA Transfer Kit**, midi, nitrocellulose
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, LF PVDF
1704275	**Trans-Blot Turbo RTA Transfer Kit**, midi, LF PVDF

Visit bio-rad.com/StainFreeWB for more information.

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