# Bio-Rad<sup>®</sup> **V3** Western Workflow™

### Save time, identify problems earlier, and have more confidence in your western blot results.

Compared to conventional methods, the stain-free technology coupled with V3 Western Workflow allows you to generate western blot results faster with more reliability and accuracy.

Workflow	Benefit
Electrophoresis	Run gels in as little as 15 min
Visualize protein separation 2 Stain-free image of pre-transfer gel	<ul> <li>Visualize separation for all lanes in 1 min</li> <li>Coomassie-like performance with no background variability and no staining/destaining</li> </ul>
Transfer 3	<ul><li>Efficient and uniform protein transfer in 3 min</li><li>Throughput: transfer 4 mini-gels at once</li></ul>
Measure transfer efficiency	Quickly assess transfer efficiency
Verify transfer efficiency	Verify quality of transfer for all lanes in 2 min
Antibody incubation	on ~5 hr
Blot detection	<ul> <li>Detect protein of interest using Clarity<sup>™</sup> western ECL substrate</li> <li>Sensitive</li> <li>Long signal duration</li> </ul>
Validate data by normalization and analysis         Image: state of the state	<ul> <li>Use stain-free blot image as total protein loading control</li> <li>No need to strip and reprobe</li> <li>Use the entire protein in a lane (no need to rely on housekeeping proteins)</li> <li>Reliable and accurate quantitation</li> </ul>

### FAQs

#### How does stain-free technology work?

The embedded compound in stain-free gels is activated by Bio-Rad imagers to provide a fluorescence signal sensitivity that is equivalent to Coomassie staining. Image stain-free gels, post-electrophoresis, with a Bio-Rad imager by selecting **stain-free gel** in Image Lab<sup>™</sup> software.

## 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 experimental conditions.
- 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 www.bio-rad.com.

Bulletin 6434	Western Blot Normalization Using Image Lab Software
Bulletin 6390	General V3 Western Workflow Blotting Protocol
Bulletin 6360	A Method for Greater Reliability in Western Blot Loading Controls: Stain-Free Total Protein Quantitation
Bulletin 6351	V3 Stain-Free Technology Publications

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

Bulletin 2895 Protein Blotting Guide www.bio-rad.com/tech/westernblotdoctor

### **Ordering Information**

Catalog #	Description							
Protein S	tandards	TGX Stain-Free <sup>™</sup> Pre	cast Gels					
161-0373	Precision Plus Protein <sup>™</sup> All Blue Standards							
161-0363	Precision Plus Protein Unstained Standards		0.4.14/-11	10 14/-11				
161-0385	Precision Plus Protein <sup>™</sup> WesternC <sup>™</sup> Pack	Description	8+1-Well 30 µl	10-Well 30 μl	10-Well 50 µl	12-Well 20 µl	15-Well 15 µl	IPG Well 7 cm IPG Strip
Buffers		· · · · · · · · · · · · · · · · · · ·	· ·		50 μi	20 μι	τομι	
161-0732	10x Tris/Glycine/SDS	Mini-PROTEAN TGX S						
161-0747	4x Laemmli Sample Buffer	7.5% Resolving Gel	456-8029	456-8023	456-8024	456-8025	456-8026	456-8021
Electroph	noresis Cell	10% Resolving Gel	456-8039	456-8033	456-8034	456-8035	456-8036	456-8031
165-6001		12% Resolving Gel	456-8049	456-8043	456-8044	456-8045	456-8046	456-8041
100 0001	power cables, 3 sample loading guides	4–15% Resolving Gel	456-8089	456-8083	456-8084	456-8085	456-8086	456-8081
165-8004		4–20% Resolving Gel	456-8099 456-8109	456-8093 456-8103	456-8094	456-8095 456-8105	456-8096 456-8106	456-8091
	vertical electrophoresis system, includes electrode assembly,	8–16% Resolving Gel Any kD Resolving Gel	456-8109	456-8103	456-8104	456-8105	456-8106	456-8101 456-8121
	campanion running module, tank, lid with power cables, mini				456-8124			
	cell buffer dam	All formats are available	in 10-packs	(catalog numb	ers listed) or 2	-packs (add a	n "S" to the e	nd of the
Blotting S	Svetom	catalog number listed).						
170-4155	-							
110 4100	includes base, 2 cassettes to hold 1–2 midi or up to 4 mini							
	blotting sandwiches, blot roller, and starter consumable kit							
170-4156	Trans-Blot Turbo Transfer Pack, mini, PVDF, pkg of 10		0453A	anuar (1925)	er   405  **			e
170-4157	Trans-Blot Turbo Transfer Pack, midi, PVDF, pkg of 10		100000000					
170-4158	Trans-Blot Turbo Transfer Pack, mini, nitrocellulose, pkg of 10		12+2-W				p+2-Well*	IPG+1-Well*
170-4159	Trans-Blot Turbo Transfer Pack, midi, nitrocellulose, pkg of 10	Description	45 µ	I 30	µl 15	μΙ	800 µl	11 cm IPG Strip
170-4270	Trans-Blot Turbo RTA Transfer Kit, mini, nitrocellulose	Criterion TGX Stain-Fi	ree Precast (	Gels**				
170-4271	Trans-Blot Turbo RTA Transfer Kit, midi, nitrocellulose	7.5% Gel	567-80	23 567-8	3024 567-	8025	_	_
170-4272	Trans-Blot Turbo RTA Transfer Kit, mini, PVDF	10% Gel	567-80	33 567-8	8034 567-	8035	_	
170-4273								-
	Trans-Blot Turbo RTA Transfer Kit, midi, PVDF	12% Gel	567-80	43 567-8	3044 567-	8045	—	_
170-4274	Trans-Blot Turbo RTA Transfer Kit, midi, PVDF Trans-Blot Turbo RTA Transfer Kit, mini, LF PVDF	12% Gel 18% Gel	567-80 567-80				— 67-8072	  567-8071
170-4274 170-4275				73 567-8	3074 567-	8075 5	— 67-8072 67-8082	  567-8071 567-8081
170-4275	Trans-Blot Turbo RTA Transfer Kit, mini, LF PVDF Trans-Blot Turbo RTA Transfer Kit, midi, LF PVDF	18% Gel	567-80	73 567-8 83 567-8	3074 567- 3084 567-	8075 5 8085 5		
	Trans-Blot Turbo RTA Transfer Kit, mini, LF PVDF Trans-Blot Turbo RTA Transfer Kit, midi, LF PVDF Systems	18% Gel 4–15% Gel 4–20% Gel 8–16% Linear Gradient	567-80 567-80 567-80 567-81	73 567-8 83 567-8 93 567-8 03 567-8	3074         567-           3084         567-           3094         567-           3104         567-	8075         5           8085         5           8095         5           8105         5	67-8082 67-8092 67-8102	567-8081 567-8091 567-8101
170-4275 Imaging \$	Trans-Blot Turbo RTA Transfer Kit, mini, LF PVDF Trans-Blot Turbo RTA Transfer Kit, midi, LF PVDF Systems Gel Doc <sup>™</sup> XR+ System with Image Lab Software, PC or Mac,	18% Gel 4–15% Gel 4–20% Gel 8–16% Linear Gradient 10–20% Linear Gradient	567-80 567-80 567-80 567-81 567-81	73 567-8 83 567-8 93 567-8 03 567-8 13 567-8	3074         567-           3084         567-           3094         567-           3104         567-           3114         567-	8075 5 8085 5 8095 5 8105 5 8115 5	67-8082 67-8092 67-8102 67-8112	567-8081 567-8091 567-8101 567-8111
170-4275 Imaging \$	Trans-Blot Turbo RTA Transfer Kit, mini, LF PVDF Trans-Blot Turbo RTA Transfer Kit, midi, LF PVDF Systems	18% Gel 4–15% Gel 4–20% Gel 8–16% Linear Gradient	567-80 567-80 567-80 567-81	73 567-8 83 567-8 93 567-8 03 567-8 13 567-8	3074         567-           3084         567-           3094         567-           3104         567-           3114         567-	8075 5 8085 5 8095 5 8105 5 8115 5	67-8082 67-8092 67-8102	567-8081 567-8091 567-8101

\* Reference wells accommodate 15 µl of markers/standards

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

#### **Detection Reagents**

170-5060 Clarity Western ECL Substrate, 200 ml

Mac, includes darkroom, UV transilluminator, epi-white illumination

standard filter, camera, power supply, cables, Image Lab software

170-5061	Clarity Western ECL Substrate, 500 ml
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