

Western Blotting Protocol: Mini-PROTEAN TGX Stain-Free Gel Run on the Trans-Blot Turbo Transfer System Using EveryBlot Blocking Buffer and Clarity and Clarity Max Western ECL Substrates

Protocol

Western Blotting

Bulletin 7442

Introduction

This easy protocol includes acceptable ranges for each applicable step. This protocol can be printed and used at the bench as a checklist for tracking the actual conditions used.

Electrophoresis

✓ Step	Instructions	Actual Conditions	Low End of Range	Ideal Conditions	High End of Range
1	Add 4x or 2x Laemmli buffer to the sample for a final concentration of 1x. Prepare 50% more than you intend to load.				
2	Heat at 95°C for 5 min.		70 2	95°C 5 min	100 10
3	Centrifuge at 10,000 x g for 5 min.		5,000 2	10,000 x g 5 min	16,000 30
4	Insert the gel and fill the gel apparatus to fill line (550 ml for one to two gels and 800 ml for three to four gels) with running buffer.				
5	Load 10 µl protein ladder/molecular weight standard.		1	10 µl	25
6	Load the gel with desired samples.				
7	Run the gel at 300 V until the dye reaches the bottom of the gel.		100	300 V	350
8	Remove the gel from the cassette and place on the imaging tray.				
9	Activate the Stain-Free dye and acquire a gel image.				

Transfer

✓ Step	Instructions	Actual Conditions	Low End of Range	Ideal Conditions	High End of Range
1	Assemble the gel and membrane inside the Trans-Blot Turbo Transfer System cassette.				
2	Run the transfer at 2.5 A for 7 min.		5	7 min	10
3	Rinse the membrane with Tris buffered saline (TBS) and place on the blot tray.				
4	Acquire a blot image.				

Blocking

✓ Step	Instructions	Actual Conditions	Low End of Range	Ideal Conditions	High End of Range
1	Immerse the membrane in 10 ml of EveryBlot Blocking Buffer.				
2	Place on a rocker and block at room temperature (RT) for 5 min.		3	5 min	30

BIO-RAD

Primary Immunodetection

✓ Step	Instructions	Actual Conditions	Low End of Range	Ideal Conditions	High End of Range
1	Dilute the antibody/antibodies in 10 ml of EveryBlot Blocking Buffer. Dilution ratio: 1: _____				
2	Place on a rocker and incubate at RT for 1 hr.		4°C 0.5	RT 1 hr	30°C 12
3	Wash with 10 ml of TBS with 0.05% Tween 20 for 5 min with agitation.		0.01 3	TBS with 0.05% Tween 20 5 min	0.1 10
4	Repeat step 3 for a total of five washes.		3	5 cycles	7

Secondary Immunodetection

✓ Step	Instructions	Actual Conditions	Low End of Range	Ideal Conditions	High End of Range
1	Dilute the antibody/antibodies in 10 ml of EveryBlot Blocking Buffer. Dilution ratio: 1: _____				
2	Place on a rocker and incubate at RT for 1 hr.		4°C 0.5	RT 1 hr	30°C 12
3	Wash with 10 ml of TBS with 0.05% Tween 20 for 5 min with agitation.		0.01 3	TBS with 0.05% Tween 20 5 min	0.1 10
4	Repeat step 3 for a total of six washes.		4	6 cycles	8

Detection

✓ Step	Instructions	Actual Conditions	Low End of Range	Ideal Conditions	High End of Range
1	Prepare 7 ml of Clarity or Clarity Max Western ECL Substrate by mixing together 3.5 ml of each part in the kit.		3	7 ml	10
2	Add the prepared substrate to the membrane and incubate for 5 min.		1	5 min	10
3	Acquire a series of images until saturation of the target band(s) is observed.				
4	Acquire a final image with no saturated bands. Filename: _____				

Signed _____ Date _____

Countersigned _____ Date _____

Go to bio-rad.com/WesternResources for more information, tips, tricks, and troubleshooting.

BIO-RAD and TRANS-BLOT are trademarks of Bio-Rad Laboratories, Inc. in certain jurisdictions. All trademarks used herein are the property of their respective owner.

TGX Stain-Free Precast Gels are covered by U.S. Patent Numbers 7,569,130 and 8,007,646.

Clarity Max Western ECL Substrate is manufactured by Cyanagen Srl and is the subject of patent application numbers US7855287, EP1950207, US9040252, AU2011202658, CA2742025, US8129136, and EP1962095, together with other equivalent granted patents and patent applications in other countries like CN102313732.



**Bio-Rad
Laboratories, Inc.**

Life Science
Group

Website bio-rad.com USA 1 800 424 6723 Australia 61 2 9914 2800 Austria 00 800 00 24 67 23 Belgium 00 800 00 24 67 23 Brazil 4003 0399
Canada 1 905 364 3435 China 86 21 6169 8500 Czech Republic 00 800 00 24 67 23 Denmark 00 800 00 24 67 23 Finland 00 800 00 24 67 23
France 00 800 00 24 67 23 Germany 00 800 00 24 67 23 Hong Kong 852 2789 3300 Hungary 00 800 00 24 67 23 India 91 124 4029300
Israel 0 3 9636050 Italy 00 800 00 24 67 23 Japan 81 3 6361 7000 Korea 82 2 3473 4460 Luxembourg 00 800 00 24 67 23
Mexico 52 555 488 7670 The Netherlands 00 800 00 24 67 23 New Zealand 64 9 415 2280 Norway 00 800 00 24 67 23 Poland 00 800 00 24 67 23
Portugal 00 800 00 24 67 23 Russian Federation 00 800 00 24 67 23 Singapore 65 6415 3188 South Africa 00 800 00 24 67 23
Spain 00 800 00 24 67 23 Sweden 00 800 00 24 67 23 Switzerland 00 800 00 24 67 23 Taiwan 886 2 2578 7189 Thailand 66 2 651 8311
United Arab Emirates 36 1 459 6150 United Kingdom 00 800 00 24 67 23

