

Western Blotting Protocol: Mini-PROTEAN TGX Stain-Free Gel Run on the Trans-Blot Turbo Transfer System Using 1x Tris Buffered Saline (TBS) with 1% Casein and Fluorescence Detection Protocol

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 1x 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 1x TBS with 1% casein.				
2	Place on a rocker and block at room temperature (RT) for 1 hr.		0.5	1 hr	3

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 1x TBS with 1% casein. 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 1x TBS with 0.05% Tween 20 for 5 min with agitation.		0.01 3	1x 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 1x TBS with 0.05% Tween 20. 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 1x TBS with 0.05% Tween 20 for 5 min with agitation.		0.01 3	1x 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	Acquire a series of images until saturation of the target band(s) is observed.				
2	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.

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