Western Blotting Protocol: Mini-PROTEAN TGX Stain-Free Gel Run on a Tank 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	Equilibrate the transfer buffer to the appropriate temperature.		4	25°C	30
	2	Equilibrate the membrane, filter paper, and fiber pads in transfer buffer for 20 min.				
	3	Prepare the gel sandwich and place the cassette into the module.				
	4	Add cooling unit, stir bar, and transfer buffer.				
	5	Run the transfer at 100 V for 1 hr.		5 0.5	100 V 1 hr	200 16
	6	Rinse the membrane with 1x TBS and place on the blot tray.				
	7	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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Bio-Rad Laboratories, Inc.

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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*
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