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

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 Tris buffered saline (TBS) and place on the blot tray.				
	7	Acquire a blot image.				

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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

Primary Immunodetection

✔	Step	Instructions	Actual Conditions	Low End of Range	Ideal Conditions	High End of Range
	1	Dilute the antibody 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 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.

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