

Western Blotting Protocol: Criterion 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 (400 ml for one gel and 800 ml for two 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 20 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 20 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 20 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 20 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 20 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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 TGX Stain-Free Precast Gels are covered by U.S. Patent Numbers 7,569,130 and 8,007,646.



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