

## WESTERN BLOT PROTOCOL - STANDARD

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*Western blot standard protocol optimized for Triple A Polyclonals and PrecisA Monoclonals from Atlas Antibodies.*

### ELECTROPHORESIS AND BLOTTING

#### SAMPLE PREPARATION

Protein samples (selected tissue lysates, cell lysates or over-expression lysates) are mixed with Laemmli buffer (to a final loading concentration of 2% SDS, 10% glycerol, 0.002% bromophenol blue, 0.0625 M TrisHCl), supplemented with DTT to a final concentration of 50 mM, and incubated in 95°C for 5 min.

#### PROCEDURE

1. Protein samples are loaded onto Criterion TGX Precast Gels, 4–20% polyacrylamide (Bio-Rad, Hercules, CA, USA). The electrophoresis is run according to manufacturer's protocols
2. The proteins are transferred from the gels to PVDF membranes through semi-dry transfer using Trans-Blot® Turbo transfer system (Bio-Rad, Hercules, CA, USA) according to manufacturer's protocol.

### IMMUNODETECTION

All incubation and wash steps are performed at room temperature and with agitation.

#### PROCEDURE

1. Dried membranes from previous steps are activated in methanol for 20 seconds. To prevent non-specific background binding of the primary and/or secondary antibodies to the membrane, membranes are blocked in milk-based blocking buffer (5% (w/v) non-fat dried milk in TBS with 0.1% (v/v) Tween20) for 45 min.
2. The primary antibody is diluted in blocking buffer and incubated with the blocked membranes for 1 h.

**NOTE:** *The recommended working dilution of the primary antibody is to be considered as a guideline only. Optimal dilution must be determined by the user.*

3. To remove residual primary antibody, the membranes are washed 3 x 5 min in TBST (TBS with 0.1% (v/v) Tween20).
4. The secondary antibody (for monoclonal antibodies: HRP-conjugated Goat Anti-Mouse Immunoglobulin; for polyclonal antibodies: HRP-conjugated Swine Anti-Rabbit Immunoglobulin, Dako, Glostrup, Denmark) is diluted 1:3000 in blocking buffer and incubated with the membranes for 30 min.
5. To remove residual secondary antibody, the membranes are washed 4 x 5 min in TBST.
6. The membranes are incubated with detection reagent (Immobilon Western Chemiluminescent HRP Substrate, Millipore Corporation, Billerica, MA, USA) for 1 min.
7. The image is captured using a CCD camera.

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