WESTERN BLOT PROTOCOL - STANDARD

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, 0.005% 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 30 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 membran- es 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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