

Western Blot Protocol

Please see if a specific Atlas antibody is verified for Western blotting under "Tested Applications" in the Product Datasheet. The Product Datasheet is viewed by clicking on the product name or product number in our online store; atlasantibodies.com/store/list.

Western blot analysis of total protein lysates from liver and tonsil, two cell lines and human plasma using the Atlas antibodies are performed as described in the following protocol.

Electrophoresis and Electroblotting

Protein samples (i.e. total protein lysates from selected human tissues, cell lines and human plasma) to be examined are separated by SDS-PAGE on appropriate gels such as Criterion Precast SDS-PAGE Gels (10-20%, Bio-Rad, Hercules, CA, USA) and transferred to a nitrocellulose or PVDF membrane using an electroblotting apparatus according to the manufacturer's protocols. Electroblotting onto PVDF membranes is performed under wet conditions in a Trans Blot Plus Cell Tank (Bio-Rad, Hercules, CA, USA) for approximately 70-80 minutes at 100V.

Immunoblotting Procedure

All washes are performed at room temperature (RT) on a shaker.

1. PVDF-membranes are pre-soaked in methanol. Non-specific sites are blocked in 5% non-fat dried milk in TBS-Tween (0.5% (v/v) Tween 20, pH7.5; blocking buffer) for 45 minutes at RT or overnight at 4°C.
2. The membrane is briefly washed in TBS-Tween (10 mM Tris, 150 mM NaCl, 0.05% (v/v) Tween 20, pH7.5; wash buffer).
3. The primary antibody is diluted in blocking buffer and the membrane is incubated for 1 hour at RT.
4. The membrane is washed for 4 x 5 minutes with large volumes of wash buffer.
5. The secondary antibody conjugated to horseradish peroxidase (HRP, Dako, Glostrup, Denmark) is diluted 3000 fold in 5 ml blocking buffer and the membrane is incubated for 1 hour at RT.
6. The membrane is washed as after incubation with the primary antibody (see step 4).
7. Excess wash buffer is drained from the membrane, which is subsequently placed in detection reagent (Immobilon Western Chemiluminescent HRP Substrate, Millipore Corporation, Billerica, MA, USA) and incubated for 30 seconds at RT.
8. Excess detection reagent is drained off and a CCD-camera is used for detection and to capture a digital image.

Antibody dilution interval: 1:250 – 1:500

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