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Product Information

Prestige Antibodies®
Powered by ATLAS ANTIBODIES

Western Blot Procedure

Product Description
Some Prestige Antibodies® are verified as primary reagents for Western blot analysis using the described procedure.

Preparation Instructions
Wash Buffer – 10 mM Tris-HCl with 150 mM NaCl and 0.05% (v/v) TWEEN® 20, pH 7.5

Blocking Buffer - 10 mM Tris-HCl with 150 mM NaCl, 5% non-fat dried milk, and 0.5% (v/v) TWEEN 20, pH 7.5

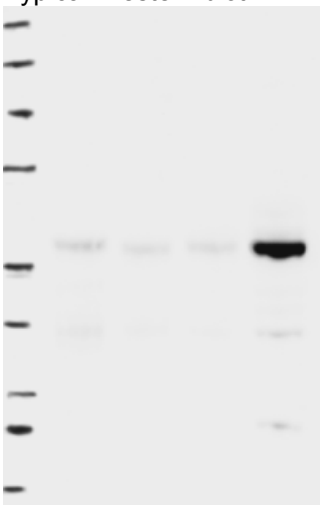
Electrophoresis and Electroblotting
Protein samples to be examined, such as human plasma and lysates from selected human tissues and cell lines, are separated by SDS-PAGE on an appropriate gel, e.g., Tris-HCl gel, 10–20%. An average of 20 µg of total protein is loaded per lane onto the gel. The protein is transferred onto a PVDF or nitrocellulose membrane by electroblotting. After transfer, the membrane is completely dried to increase the protein retention prior to immunoblotting.

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

Antibody dilution: 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.

1. PVDF membranes are soaked in methanol.
2. Non-specific sites are blocked on both PVDF or nitrocellulose membranes with Blocking Buffer for 45 minutes at RT or overnight at 4 °C.
3. The membrane is quickly rinsed in Wash Buffer followed by incubation for 1 hour at RT in the primary antibody diluted in 5 ml of Blocking Buffer.
4. The membrane is rinsed 4 times, 5 minutes each wash, with large volumes of Wash Buffer.
5. The secondary antibody horseradish peroxidase conjugate is diluted 3,000-fold in 5 ml of Blocking Buffer and the membrane is incubated for 1 hour at RT.
6. The membrane is washed as in step 4.
7. Excess Wash Buffer is drained from the membrane, which is then placed in a chemiluminescent substrate solution and incubated for 30 seconds at RT in darkness.
8. Excess detection reagent is drained off and a CCD-camera is used for detection and capture of a digital image

Figure 1.
Typical Western blot



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