

Prestige Antibodies™

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ANTIBODIES

Western Blot Procedure

Product Description

Some HPA antibodies are verified as primary reagents for Western blot analysis using the described procedure.

Preparation Instructions

Electrophoresis and Electroblothing

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., Bis-Tris gel, 4–12%. An average of 20 µg of total protein is loaded per lane onto the gel. Electroblothing onto a PVDF membrane is performed using a semi-dry blotter. 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. After wetting the membrane with methanol, the membrane is briefly washed in TBST (10 mM Tris, with 150 mM NaCl and 0.05% (v/v) TWEEN® 20, pH 7.5).
2. Non-specific sites are blocked in blocking buffer (5% non-fat dried milk in TBST with 0.1% (v/v) TWEEN 20, pH 7.5) for 45 minutes at RT or overnight at 4 °C.
3. The membrane is quickly rinsed in wash buffer (TBST with 0.1% (v/v) TWEEN 20, pH 7.5), followed by incubation for 1 hour at RT in the primary antibody diluted in 5 ml of blocking buffer.
4. The membrane is quickly rinsed twice in large volumes of wash buffer followed by extended washing with wash buffer, 3 times for 10 minutes each.
5. The secondary antibody horseradish peroxidase conjugate is diluted in 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 5–10 minutes 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

Results

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Figure 1.
Typical Western blot

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