

Protocol: Western Blot

Please see if a specific HPA Antibody is verified for Western blotting under "Tested Applications" in the product data sheet. The product data sheets are viewed by clicking on the product name or product number in our online store <http://www.atlasantibodies.com/store/list>. HPA Antibodies verified for Western blotting have a Western blot image included in the product data sheet.

Western blot analysis for Tissues Western applications using HPA antibodies verified as primary reagents for Western blotting are performed as described in the following protocol.

Electrophoresis and Electroblothing

Protein samples such as lysates from human plasma, selected human tissues and cell lines to be examined are separated by SDS-PAGE on an appropriate gel (e.g. NuPAGE Bis-Tris SDS-PAGE Gel, 4-12%, Invitrogen). An average amount of 20µg total protein per lane is loaded onto the gel. Electroblothing onto a PVDF membrane is performed under semi-dry conditions in a TE 77 Semi-dry transfer unit (Amersham Biosciences) for approximately 70 minutes according to the manufacturer's recommendations. After transfer, the membrane is completely dried to increase the protein retention prior to immunoblotting.

Immunoblotting Procedure

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

1. After reactivation in methanol, the membrane is briefly washed in TBS-T (10mM Tris, 150mM NaCl, 0.05% (v/v) Tween 20, pH7.5).
2. Non-specific sites are blocked in blocking buffer (5% non-fat dried milk in TBS-T, 0.1% (v/v) Tween 20, pH7.5) for 45 minutes at RT or overnight at 4°C.
3. The membrane is quickly rinsed in wash buffer (TBS-T, 0.1% (v/v) Tween 20, pH7.5) followed by incubation for 1 hour at RT in the primary antibody diluted in 5ml blocking buffer.
4. The membrane is quickly rinsed twice in large volumes of wash buffer followed by extended washing for 3 x 10 minutes.
5. The secondary antibody conjugated to horseradish peroxidase (HRP, Dako) is diluted 3000 fold in 5ml blocking buffer and the membrane is incubated for 1 hour at RT.
6. The membrane is washed as above (see step 4).
7. Excess wash buffer is drained from the membrane, which is subsequently placed in detection reagent (SuperSignal West Dura Extended duration Substrate, Pierce) 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 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.

Warranty: The products supplied by Atlas Antibodies AB are warranted to meet stated product specifications and to conform to label descriptions when used and stored properly. Unless otherwise stated, this warranty is limited to one year from date of sales for products used, handled and stored according to Atlas Antibodies AB's instructions. Atlas Antibodies AB's sole liability is limited to replacement of the product or refund of the purchase price. All products are supplied for research use only. They are not intended for medicinal, diagnostic or therapeutic use. No products from Atlas Antibodies AB may be resold, modified for resale or used to manufacture commercial products without prior written approval from Atlas Antibodies AB.

Atlas Antibodies AB, AlbaNova University Center, SE-106 91 Stockholm, Sweden. Phone: +46-8-54 59 58 50, Fax: +46-8-54 59 58 51
E-mail: contact@atlasantibodies.com, Web: www.atlasantibodies.com.

Rev. August, 2006