

## Anti-RhoA Monoclonal Antibody

Cat. # ARH03

Upon arrival, store at 4°C (desiccated)

See datasheet for storage after reconstitution

### Storage and Reconstitution

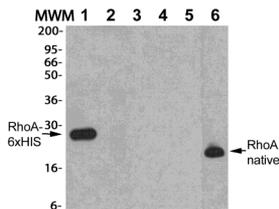
The recommended storage conditions for the lyophilized material is 4°C and <10% humidity. Under these conditions the protein is stable for 1 year. Lyophilized protein can also be stored desiccated at -70°C.

Upon receipt, the product tube should be briefly centrifuged to collect the powder at the bottom of the tube. Resuspend each tube in 200 µl of PBS plus 0.1% sodium azide. Anti-RhoA antibody should be stored at 4°C and is stable for 6 months. THE ANTI-BODY SHOULD NOT BE FROZEN.

Resuspend the platelet extract control protein in 500 µl of 1x SDS-PAGE sample loading buffer for a final concentration of 2 mg/ml, aliquot into 10 X 50 µl amounts (100 µg each), and store at -70°C.

### Material

RhoA antibody is provided as an affinity purified mouse monoclonal antibody. The antibody has been raised against a peptide sequence of RhoA (amino acids 120-150). This antibody is specific for RhoA. Human platelet extract is provided as the positive control protein. Anti-RhoA is supplied as a lyophilized powder.



**Figure 1. Western blot analysis of anti-RhoA antibody.**

Recombinant small G-proteins and tissue extracts were separated by SDS-PAGE and transferred to PVDF membrane. Anti-RhoA antibody was diluted to 250 ng/ml (1:1000) in PBST and Western analysis was as described in the Methods section. Lane 1; 25 ng RhoA-6xHIS, Lane 2; 250 ng RhoB-6xHIS, Lane 3; 250 ng RhoC-6xHIS, Lane 4; 250 ng Rac1-6xHIS, Lane 5; 250 ng Cdc42-6xHIS, Lane 6; 50 µg human platelet extract. Note: RhoA-6xHIS runs at 28kDal whereas the native RhoA in platelet extract runs at 23kDal.

### Methods

#### Western blot analysis

#### Reagents:

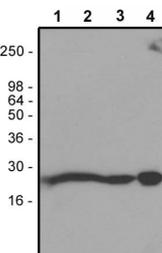
- 1) Anti-RhoA (Cat. # ARH03)
- 2) SDS-PAGE and Western blot equipment
- 3) PVDF or Nitrocellulose membrane (Millipore Inc.)
- 4) Transfer Buffer (ice cold): 25 mM Tris-HCl, pH 8.3; 192 mM glycine, 15% methanol
- 5) TBST: 10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20
- 6) Blotto: 5% non-fat dry milk in TBST
- 7) HRP-conjugated goat anti-mouse antibody (Jackson labs)
- 8) Chemiluminescence detection reagents (ECL, Amersham Biosciences)

#### Method:

- 1) Separate protein samples on a 4-20% SDS PAGE gel until the dye-front reaches the bottom of the gel.
- 2) Electrobolt the proteins onto PVDF or Nitrocellulose membrane for 45 min at 300 mA only with fresh transfer buffer (do not exceed time limit because Rho proteins will pass through the membrane).
- 3) Block the membrane in Blotto for 60 min at room temperature.
- 4) Probe with 250 ng/ml (1:1000 dilution) of ARH03 in TBST for 1 h. A 1:500 dilution of antibody can be used if protein samples have a low abundance of RhoA.
- 5) Wash the membrane three times with TBST for 5 min each.
- 6) Probe with 1:40,000 dilution of the anti-mouse-HRP antibody in TBST for 1 h. A 1:20,000 dilution can be used if protein samples have a low abundance of RhoA.
- 7) Wash the membrane six times with TBST for 5 min each.
- 8) Process the blots for chemiluminescence detection using a high potency reagent such as ECL.
- 9) Typical results are shown in Figures 1 and 2.

**Figure 2. Western blot of cell extracts probed with anti-RhoA antibody (Cat. # ARH03).**

Chemiluminescence detection of RhoA in cell extracts (50 µg each) of rat NRK cells (lane 1), human HeLa cells (lane 2), bovine brain extract (lane 3), and human platelet cell extract (lane 4). The RhoA band is indicated at approx. 25kDa (see arrow). The blot was probed with a 250 ng/ml (1:1000) dilution of anti-RhoA antibody. Molecular weight markers are from Invitrogen.



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