

Anti-RhoA Mouse MAb

Cat. # ARH04

Upon arrival, store at 4°C (desiccated)
 See datasheet for storage after reconstitution

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|--------------------------------|-------------------------------|
| Form: | Lyophilized powder |
| Amount of material: | 2 x 200 µl when reconstituted |
| Validated applications: | WB |
| Species reactivity: | Mammalian, reptile, avian |
| Host/Isotype: | Mouse/IgG1 |
| Clone: | 7F1.E5 |

Background Information

RhoA (mol. wt. 21 kDa) belongs to the Rho-family of small G-proteins (1). The Rho family consists of at least 22 members, the most extensively characterized of which are the Rac1, RhoA and Cdc42 proteins (2). In common with all other small G-proteins, Rho family proteins act as molecular switches that transmit cellular signals through an array of effector proteins. The family mediates a diverse number of cellular responses including cytoskeletal reorganization (3), regulation of transcription (4), apoptosis (5) and neuronal morphology (6).

Proteins within the Rho-family share 40-95% amino acid identity within their GTPase domains (1).

Material

ARH04 anti-RhoA antibody is a mouse monoclonal antibody that specifically recognizes RhoA. ARH04 was raised against the peptide CDEHTRRE-LAKMKQEPVKPEEGRD conjugated to KLH. The antibody can detect 2 ng of recombinant RhoA and can detect endogenous RhoA in 4 µg of platelet cell extract. ARH04 is purified by protein G affinity chromatography and is supplied as a lyophilized white powder. Each Lot of antibody is quality controlled to provide a high batch to batch consistency. The Lot specific µg per tube can be found in the Lot specific COA documents at www.cytoskeleton.com. ARH04 shows high specificity to RhoA and does not cross-react with the closely related RhoC in a western blot (Figure 1).

Storage and Reconstitution

Shipped at ambient temperature. The lyophilized protein can be stored desiccated at 4°C for 6 months. For reconstitution, the product tube should be briefly centrifuged to collect the powder at the bottom of the tube. Reconstitute each tube in 200 µl of PBS and store at 4°C. When stored and reconstituted as described, the product is stable for 6 months at 4°C. NOTE: We recommend adding an antibacterial such as sodium azide (0.02% final concentration) to prevent bacterial contamination of the antibody stock. **THE ANTIBODY SHOULD NOT BE FROZEN.**

Applications

Western Blot (WB) Applications

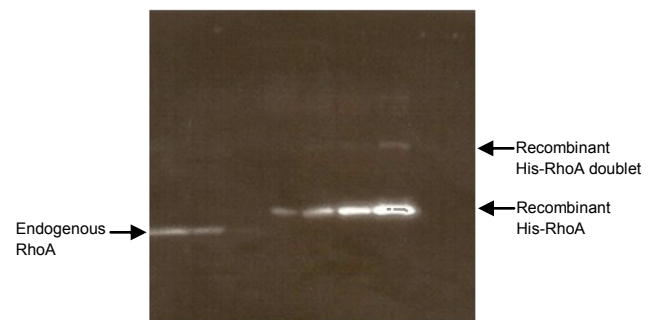
Use as indicated in method at 1:500 - 1:1000 dilution, sufficient for 200-400 ml of working strength Ab.

Western Blot Method:

- Run protein samples and control samples on SDS-PAGE.
- Equilibrate the gel in Western blot buffer (25 mM Tris pH 8.3, 192 mM glycine, 5% methanol) for 15 min at room temperature prior to electro-blotting.
- Transfer the protein to a PVDF membrane for 45 minutes at 75V.
- Wash the membrane once with TBST for 10 minutes (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20).
- Allow the membrane to air dry for 20-30 minutes at room temperature.
- Transfer membrane to TBST at room temperature for 15 minutes to rehydrate the membrane. It is convenient, at this point, to leave the membrane in TBST overnight at 4°C.
- Block the membrane surface with 5% nonfat-dry milk in TBST for 30 min at room temperature with constant agitation.
- Incubate the membrane with a 1:500 to 1:1000 dilution of anti-RhoA antibody diluted in TBST for 1-2 h at room temperature or overnight at 4°C with constant agitation.
- Rinse the membrane in 50 ml TBST for 1 min.
- Incubate the membrane with an appropriate dilution (eg. 1:20,000) of anti-mouse secondary antibody (eg. goat anti-mouse HRP conjugated IgG from Jackson Labs., Cat. # 115-035-068) in TBST for 30 min.

- Wash the membrane 6 times in TBST for 10 min each.
- Use an enhanced chemiluminescence detection method to detect the RhoA signal (eg. SuperSignal West Dura Extended Duration Substrate; ThermoFisher). Typical results are shown in figure 1.

Figure 1: Western Blot: Demonstration of ARH04 sensitivity and specificity



Legend: Recombinant small G-proteins and human platelet extract were separated by SDS-PAGE and transferred to a PVDF membrane according to the method given in this datasheet. Anti-RhoA was diluted 1 in 500 in TBST and western analysis was performed as detailed in the Western Blot Method section. Lane 1: 40 µg platelet extract, Lane 2: 20 µg platelet extract, Lane 3: 4 µg platelet extract, Lane 4: 2 ng His-RhoA, Lane 5: 5 ng His-RhoA, Lane 6: 10 ng His-RhoA, Lane 7: 20 ng His-RhoA, Lane 8: 100 ng His-RhoC. The recombinant and native RhoA band is visible at approximately 23 kD. No signal is visible with 100 ng of His-RhoC (Lane 8). The faint bands visible above His-RhoA represent a minor species of His-RhoA doublet. Exposure time 2 minutes.

References

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