

Affinity-Purified Sheep Anti-human/mouse/rat ARHGAP1 Antibody

ORDERING INFORMATION

Catalog Number: AF6407

Lot Number: CEKB01

Size: 100 µg

Storage: -20° C

Specificity: human, mouse and rat ARHGAP1

Immunogen: *E. coli*-derived rhARHGAP1
(aa 1 - 62)

Ig Type: sheep IgG

Application: Western blot

Background

ARHGAP1 (also p50-RhoGAP and CDC42 GTPase-activating protein) is a 50 kDa member of the ARHGAP family of proteins. It is preferentially expressed in epithelium, and serves as a cytoplasmic protein that deactivates Rho-family GTPases. It does so by contributing an Arg residue (Arg282) to Cdc42 and RhoA, which activates their intrinsic GTPase activity and converts GTP-associated molecules into inactivating GDP molecules. Human ARHGAP1 is 439 amino acids (aa) in length and contains one CRAL-TRIO domain that binds small lipophilic molecules (aa 53 - 218), an SH3 binding site (aa 228 - 238) and one C-terminal RhoGAP domain (aa 244 - 431). There are phosphorylation sites at Ser51 and Tyr65. There is one potential splice form with a 24 aa substitution for aa 1 - 150 coupled to a 26 aa substitution for aa 300 - 439. Over aa 1 - 62, human ARHGAP1 shares 96% aa identity with mouse ARHGAP1.

Preparation

Sheep antibodies were raised against purified *E. coli*-derived, recombinant human ARHGAP1 (rhARHGAP1; aa 1 - 62; Accession # Q07960). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein and further purified by isolating the IgG fraction.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute the antibody in PBS containing 0.02% Na₃N.

Storage

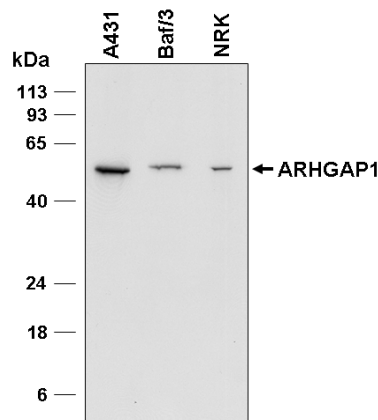
Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

The antibody detects endogenous human, mouse and rat ARHGAP1 at 50 kDa using Western blots.

Application

Western blot - An antibody concentration of 0.2 µg/mL is recommended.



Detection of ARHGAP1 with AF6407.

Lysates from human A431, mouse Baf/3 and rat NRK cells were resolved by SDS-PAGE. Following electrophoresis, proteins were transferred to an Immobilon-P membrane and immunoblotted with 0.2 µg/mL sheep anti-ARHGAP1, as described in *Protocols for Immunoblotting*.

Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk	5% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to an Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane for 1 hour at room temperature in Antibody Solution containing 0.2 µg/mL sheep anti-human/mouse/rat ARHGAP1.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated donkey anti-sheep IgG (R&D Systems, Catalog # HAF016).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagents.

Cell lysates for Western blottings - To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF and bromophenyl blue) at 2×10^6 - 1×10^7 cells per mL.

Optimal dilutions should be determined by each laboratory for each application.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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