V.5.0

Anti-Cdc42 Monoclonal Antibody
Cat. # ACD03

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

Background Information

Cdc42 (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

The anti-Cdc42 antibody is a mouse monoclonal antibody. It has been purified by affinity chromatography over a protein G column. The antibody was raised against a peptide sequence of human Cdc42 (amino acids 128-138). The antibody specifically recognizes Cdc42 in a wide range of species, including mammalian, reptile and avian sources. Quality Control analysis has shown that the antibody does not recognize Rac1 or RhoA (see figure 1). ACD03 is supplied as a lyophilized white powder. Human platelet extract is included as a positive control.

Storage and Reconstitution

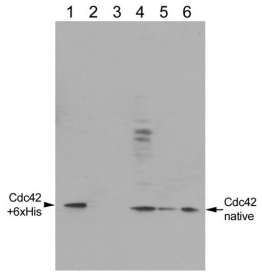
Shipped at ambient temperature. The lyophilized protein can be stored desiccated at 4°C for 6 months. For reconstitution, the product tube should be briefly centrifuged to collect the powder at the bottom of the tube . Reconstitute to 250 µg/ml in PBS (200 µl PBS per 50 µg of antibody) and store at 4°C. 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.

Resuspend the platelet extract control protein in 500 µl of 1x SDS-PAGE sample loading buffer (63 mM Tris HCl pH 6.8, 2% SDS, 2.5% β-mercaptoethanol, 0.003% bromophenol blue,10% glycerol) for a final concentration of 2 mg/ml, aliquot into 20 X 25 µl amounts (50 µg each), store at -20°C or -70°C.

Biological Activity Assay

ACD03 was analyzed by its ability to specifically recognize endogenous Cdc42 in 40 µg of human platelet extract in western blot analysis (see figure 1).

Figure 1: Western blot analysis using Anti-Cdc42 antibody



Legend: Recombinant small G-proteins and various tissue extracts were separated by SDS-PAGE and transferred to a PVDF membrane according to the method given in this datasheet. Anti-Cdc42 was diluted to 250 ng/ml in TBST plus 0.1% non-fat milk powder and western analysis was performed as detailed in the Western Blot Method section. Lane 1: 25 ng His-Cdc42, Lane 2: 250 ng His-RhoA, Lane 3: 250 ng His-Rac1, Lane 4: 40 µg 3T3 cell extract, Lane 5: 40 µg of bovine brain extract, Lane 6: 40 µg human platelet extract.

Western Blot Method:

1. Run protein samples and control samples on a 4-20% SDS-PAGE until the dye from reaches the bottom of the gel.
2. We recommend running 40 µg human platelet extract as a control for Cdc42 signal (see Figure 1 Lane 6).
3. Equilibrate the gel in Western blot buffer (See recipe below) for 15 min at room temperature prior to electro-blotting.
4. Transfer the protein to a PVDF membrane for 45 minutes at 75V.
5. Wash the membrane once with TBS (10 mM Tris-HCl pH 8.0, 150 mM NaCl).
6. Allow the membrane to air dry for 20-30 minutes at room temperature.
7. Transfer membrane to TBST (10 mM Tris-HCl ph 8.0, 150 mM NaCl, 0.05% Tween 20) at room temperature for 15 minutes to

Phone: (303) 322.2254 **Fax:** (303) 322.2257
Customer Service: cserve@cytoskeleton.com
Technical Support: tservice@cytoskeleton.com

rehydrate the membrane. It is convenient, at this point, to leave the membrane in TBST overnight at 4°C.

8. Block the membrane surface with 5% nonfat-dry milk in TBST for 30 min at room temperature with constant agitation.
9. Incubate the membrane with a 1:250 (recommended) to 1:1000 dilution of anti-Cdc42 antibody diluted in TBST plus 0.1% nonfat-dry milk for 1-2 h at room temperature or overnight at 4°C with constant agitation.
10. Rinse the membrane in 50 ml TBST for 1 min.
11. 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-1 h.
12. Wash the membrane 5 times in TBST for 10 min each.
13. Use an enhanced chemiluminescence detection method to detect the Cdc42 signal (eg. SuperSignal West Dura Extended Duration Substrate; ThermoFisher).

Recipe for Western Blot Buffer (1 L)

1 M Tris pH 8.3	25 ml (25 mM final)
Glycine	14.4 g (192 mM final)
Methanol	150 ml (15% final)
Distilled water to	1 L

Product Uses

- Specific detection of Cdc42 in a wide range of species, including mammalian, reptile and avian sources.

References

1. Wennerberg K. and Der C.J. 2004. Rho-family GTPases: it's not only Rac and Rho (and I like it). *J. Cell Sci.* **117**: 1301-1312.
2. Nobes, C.D. et al. 1995. Rho, Rac, and Cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell.* **81**, 53-62.
3. Ridley, A.J. & Hall, A. 1992. The small GTP-binding protein Rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell.* **70**, 389-399.
4. Hill, C.S. et al. 1995. The Rho family GTPases RhoA, Rac1, and Cdc42Hs regulate transcriptional activation by SRF. *Cell.* **81**, 1159-1170.
5. Subauste, M.C. et al. 2000. Rho Family Proteins Modulate Rapid Apoptosis Induced by Cytotoxic T Lymphocytes and Fas. *J. Biological Chem.* **275**, 9725-9733.
6. Chen C. et al. 2012. Cdc42: An important regulator of neuronal morphology. *Int. J. Biochem. Cell Biol.* **44**, 447-451.

Product Citations/Related Products

For the latest citations and related products please visit www.cytoskeleton.com