V 1.0

Anti-Cdc42 Mouse MAb

Cat. # ACD04-S
Lot:
Upon arrival, store at 4°C (desiccated)
See datasheet for storage after reconstitution

Form:	Lyophilized powder
Amount of material:	1 x 125 µl when reconstituted
Validated applications:	WB
Reactivity:	Mammalian, reptile, avian, amphibian, fish, insect
Host/Isotype:	Mouse/Ig2b
Clone:	93B11.3.1

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. The antibody was raised against a peptide sequence of human Cdc42 (amino acids 150-182), it specifically recognizes Cdc42 in a wide range of species, including mammalian, reptile and avian and amphibian sources. Quality Control analysis has shown that the antibody does not recognize Rac1. ACD04 is supplied as a lyophilized white powder. Each Lot of antibody is quality controlled to provide a high batch to batch consistency.

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 500 µl of PBS and store at 4°C. When stored and reconstituted as described, the product is stable for 6 months at 4°C. Bovine serum albumin is included in the product at 2 mg/ml final concentration. 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

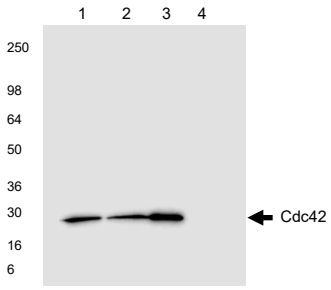
Western Blot (WB) Applications

Use as indicated in method at 1:1000 dilution, sufficient for 125 ml of working strength Ab.

Western Blot Method:

1. Run protein samples and control samples on SDS-PAGE.
2. Equilibrate the gel in Western blot buffer (25 mM Tris pH 8.3, 192 mM glycine, 15% methanol) for 15 min at room temperature prior to electro-blotting.
3. Transfer the protein to a PVDF membrane for 45 minutes at 75V.
4. Wash the membrane once with TBST for 10 minutes (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20).
5. Allow the membrane to air dry for at least 20-30 minutes at room temperature.
6. Rehydrate the membrane by briefly rinsing in methanol.
7. Transfer membrane to TBSTat room temperature to equilibrate.
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 1:1000 (recommended) to 1:2000 dilution of anti-Cdc42 antibody diluted in TBST 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.
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).

Figure 1: Western Blot: Demonstration of ACD04 sensitivity & specificity



Legend: Cell extracts were separated by SDS-PAGE and transferred to a PVDF membrane according to the method given in this datasheet. Anti-Cdc42 was diluted 1 in 1000 in TBST and western analysis was performed as detailed in the Western Blot Method section. The following lysates were run on the gel: Lane 1: 20 µg A431 cells, Lane 2: 20 µg HeLa cells, Lane 3: 20 µg porcine brain, Lane 4: 500 µg His-tagged Rac1 protein (m.wt. 21 kD). The native Cdc42 band is visible at approximately 23 kD. Exposure time 2 minutes.

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

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