

Anti-Cdc42 Mouse MAb

Cat. # ACD03

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

Form:	Lyophilized powder
Amount of material:	2 x 200 µl when reconstituted
Validated applications:	WB
Species reactivity:	Mammalian, reptile, avian
Host/Isotype:	Mouse/nd [ascites]
Clone:	4B3

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 manufactured by production of mouse ascites fluid from clone 4B3. The antibody was raised against a peptide sequence of human Cdc42 (amino acids 128-138), it 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 (see figure 1). ACD03 is supplied as a lyophilized white powder. Each Lot of antibody is quality controlled to provide a high batch to batch consistency. Lot specific information can be found in the ACD03 COA documents at www.cytoskeleton.com. 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 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.**

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.

Applications

Western Blot (WB) Applications

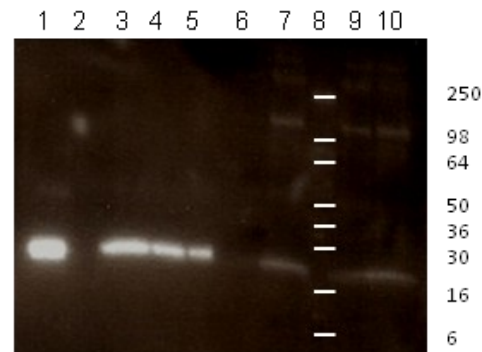
Use as indicated in method at 1:250 dilution, sufficient for 100 ml of working strength Ab.

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 1:250 (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.

- 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 5 times in TBST for 10 min each.
- 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 ACD03 sensitivity & 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-Cdc42 was diluted 1 in 250 in TBST plus 0.1% non-fat milk powder and western analysis was performed as detailed in the Western Blot Method section. Lane 1: 50 ng His-Cdc42, Lane 2: 500 ng His-Rac1, Lane 3: 20 ng His-Cdc42, Lane 4: 10 ng His-Cdc42, Lane 5: 5 ng His-Cdc42, Lane 6: 4 µg platelet extract, Lane 7: 40 µg platelet extract, Lane 8: position of molecular weight markers, Lane 9: 20 µg platelet extract, Lane 10: 50 µg platelet extract. The recombinant and native Cdc42 band is visible at approximately 23 kD. No signal is visible with 500 ng of His-Rac1 (Lane 2). Exposure time 2 minutes.

References

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- 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.
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