



Anti-Cdc42 Monoclonal Antibody

Cat. # ACD03

Lot # 016 Amount: 1 x 100 µg

Shipped at room temperature

Store at 4°C

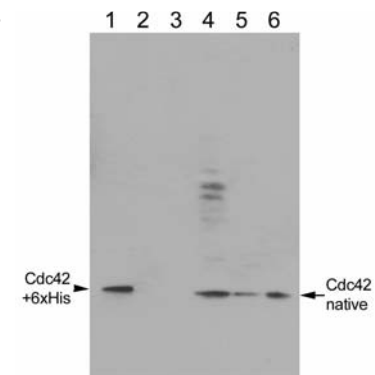
Material

Cdc42 antibody is provided as an affinity purified mouse monoclonal antibody. The antibody has been raised against a peptide sequence of human Cdc42 (amino acids 123-141). ACD03 will not cross react with a ten fold excess of RhoA, RhoB, RhoC, Rac1, Rac2 or Rac3 proteins. Non-specific bands have been observed at higher molecular weight in about 50% of cell lines tested. Non-specific bands can be minimized by following the western protocol on page 2. Anti-Cdc42 antibody is supplied as a white lyophilized powder. Human platelet extract is provided as the positive control protein which shows one band at 22kDal, it is a blue lyophilized powder.

Product Uses

Western blot	1:500 to 1:1000	This antibody is recommended for detection of Cdc42 in westerns of human, mouse, rat, and bovine extracts (Fig. 1).
Immunofluorescence	1:200	Use PBS plus 10mg/ml BSA in as blocking agent.

Figure 1. Western blot analysis of anti-Cdc42 antibody. Recombinant small G-proteins and tissue extracts were separated by SDS-PAGE and transferred to PVDF membrane. ACD03 was diluted to 250 ng/ml (1:1000) in PBST plus 0.1% non-fat milk powder and Western analysis was as described in the Methods section. Lane 1; 25 ng Cdc42-6xHIS, Lane 2; 250 ng RhoA-6xHIS, Lane 3; 250 ng Rac1-6xHIS, Lane 4; 40 µg Swiss 3T3 cell extract, Lane 5; 40 µg bovine brain extract, Lane 6; 40 µg human platelet extract. Note: Cdc42-6xHIS runs at 25kDal whereas the native Cdc42 runs at 22kDal.



Storage and Handling

Upon receipt, the product tube should be briefly centrifuged to collect the powder at the bottom of the tube. Resuspend in 400 µl of PBS plus 0.1% sodium azide. Anti-Cdc42 antibody should be stored at 4°C and is stable for 6 months. Alternatively, aliquot into 5 x 80 µl, snap freeze in liquid nitrogen and store at -70°C.

Resuspend the platelet extract control protein in 500 µl of 1x SDS-PAGE sample loading buffer for a final concentration of 2 mg/ml, aliquot into 10 X 50 µl amounts (100 µg each), and store at -70°C.

Methods

Western blot analysis

Reagents:

- 1) Anti-Cdc42 MAb (Cat. # ACD03)
- 2) SDS-PAGE and Western blot equipment
- 3) PVDF or Nitrocellulose membrane (Millipore Inc.)
- 4) Transfer Buffer (ice cold): 25 mM Tris-HCl, pH 8.3; 192 mM glycine, 15% methanol
- 5) TBST: 20 mM Tris-HCl pH 8.0, 50 mM NaCl, 0.01% Tween 20
- 6) Blotto: 5% non-fat dry milk powder in TBST
- 7) HRP-conjugated goat anti-mouse antibody (Jackson labs)
- 8) Chemiluminescence detection reagents (ECL, Amersham Biosciences)

Method:

- 1) Separate protein samples on a 4-20% SDS PAGE gel until the dye-front reaches the bottom of the gel.
- 2) Electroblot the proteins onto PVDF or Nitrocellulose membrane for 45 min at 300 mA only with fresh transfer buffer (do not exceed time limit because Rho proteins will pass through the membrane).
- 3) Block the membrane in Blotto for 60 min at room temperature.
- 4) Probe with 250 ng/ml (1:1000 dilution) of ACD03 in TBST plus 0.1% non-fat milk powder for 1 h. A 1:500 dilution of antibody can be used if protein samples have a low abundance of Cdc42.
- 5) Wash the membrane three times with TBST for 5 min each.
- 6) Probe with 1:20,000 dilution of the anti-mouse-HRP antibody in TBST for 1 h.
- 7) Wash the membrane six times with TBST for 5 min each.
- 8) Process the blots for chemiluminescence detection using a high potency reagent such as ECL.
- 9) Typical results are shown in Figure 1.

Associated Products

* Anti-Rac1 antibody (mouse monoclonal)	Cat. # ARC03
* Anti-RhoA antibody (mouse monoclonal)	Cat. # ARH03
* RhoA Activation Assay, luminescence ELISA format	Cat. # BK121
* RhoA, Activation Assay, colorimetric ELISA format	Cat. # BK124
* Rho inhibitor (cell permeable)	Cat. # CT04
* Rho Activator (Calpeptin 5 x 50 µg aliquots, culture tested)	Cat. # CN01
* Rac and Cdc Activator (EGF 5 x 100 ng aliquots, cult. tested)	Cat. # CN02
* Rac1,2,3 Activation Assay, colorimetric ELISA format	Cat. # BK125
* Rac1 Activation Assay, luminescence ELISA format	Cat. # BK126
* RhoGEF GTP Exchange Assay kit	Cat. # BK100
* RhoGAP GTPase Assay kit	Cat. # BK105
* RhoA Activation Assay Kit (pulldown format)	Cat. # BK036
* Rac Activation Assay Kit (pulldown format)	Cat. # BK035
* Cdc42 Activation Assay Kit (pulldown format)	Cat. # BK034
* Ras Activation Assay Kit (pulldown format)	Cat. # BK008
* EasyRad Phosphate Assay Kit (GTPase assay)	Cat. # BK055
* His-RhoA protein: constitutively active	Cat. # R6301
* His-RhoC protein: wild-type	Cat. # RH03
* GST-RhoA protein: wild-type	Cat. # RHG01