Datasheet for ABIN487309

anti-FZR1 antibody

2 Images

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Overview

Quantity:	0.1 mg
Target:	FZR1
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This FZR1 antibody is un-conjugated
Application:	Western Blotting (WB), Immunoprecipitation (IP)

Product Details

Immunogen:	Recombinant Human CDH1 protein. Remarks: Hybridoma was established by fusion of Mouse myeloma cell NS-2 with Balb/cmouse splenocyte
Clone:	DCS-266
Isotype:	IgG1
Cross-Reactivity (Details):	Species reactivity (tested):Human, Mouse and Rat.
Characteristics:	Synonyms: Cdh1/Hct1 homolog, CDC20-like protein 1, Fizzy-related protein homolog, FYR, FZR,KIAA1242
Purification:	Protein-A Sepharose Chromatography.

Target Details

Target:	FZR1

Target Details

Alternative Name:	FZR1 / CDH1 (FZR1 Products)
Background:	Two highly conserved WD40-repeat proteins, Cdc20 and Cdh1/Hct1, have been identified as limiting, substrate-specific regulators for anaphase-promoting complex (APC)-dependent proteolysis. Cdc20 and Cdh1 bind directly to APC and activate its cyclin ubiquitination activity. Cdc20 activates APC at the onset of anaphase, while Cdh1 activates APC from late anaphase through G1. Phosphorylation of Cdh1 by cyclin dependent kinases (Cdks), such as Cdc28, blocked Cdh1-APC interaction and APC activation, while dephosphorylation by the phosphatase Cdc14 restored this activity. Activation of the APC and cyclin degradation is required for exit from mitosis, hence, the phosphorylation of Cdc20 and Cdh1 by Cdks regulate APC activity and may control the precise progression of mitosis.Synonyms: CDC20-like protein 1, Cdh1/Hct1 homolog, FYR, FZR, Fizzy-related protein homolog, KIAA1242
Gene ID:	51343
UniProt:	Q9UM11
Pathways:	DNA Replication, Synthesis of DNA

Application Details

Application Notes:	Western Blot: 1-5 µg/mL for chemiluminescence detection system. Positive Controls: Jurkat, NIH/3T3, C2C12 and Rat-1 cells. Immunoprecipitation: 1-2 µg/200-500 µL of cell extract from 5 x 10^6 cells. . Positive Control: HeLa Cells. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user. Species Reactivity: Tested: Human, Mouse and Rat. Add. Information: This product was originally produced by MBL International.
Protocol:	SDS-PAGE & Western Blotting1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mMTris-HCl, pH 7. 2, 250 mM NaCl, 0. 1% NP-40, 2 mM EDTA, 10% glycerol) containingappropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, thensonicate briefly (up to 10 seconds). 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant toanother tube. Measure the protein concentration of the supernatant and add the Lysisbuffer to make 8 mg/mL solution. 3) Mix the sample with equal volume of Laemmli's sample buffer. 4) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1mm thick SDS-polyacrylamide gel for electrophoresis. 5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hourin a semi-dry transfer system. (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the

Application Details

	manufacture's manual for the transfer procedure. 6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH7. 2) for 1 hour at room temperature, or overnight at 4°C. 7) Incubate the membrane with primary antibody diluted with PBS, pH 7. 2 containing 1%skimmed milk as suggested in the APPLICATIONS for 1 hour at room temperature. (Theconcentration of antibody will depend on the conditions.)8) Wash the membrane with PBS (5 minutes x 6 times). 9) Incubate the membrane with the 1: 10000 POD-conjugated anti-mouse IgG diluted with1% skimmed milk (in PBS, pH 7. 2) for 1 hour at room temperature. 10) Wash the membrane with PBS (5 minutes x 6 times). 11) Wipe excess buffer from the membrane, then incubate it with appropriatechemiluminescence reagents for 1 minute. Remove extra reagent from the membrane bydabbing with a paper towel, and seal it in plastic wrap. 12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it inplastic wrap. 13) Expose to an X-ray film in a dark room for 3 minutes. 14) Develop the filmas usual. The condition for exposure and development may vary. Positive Controls for Western blotting: Jurkat, NIH/3T3, C2C12, Rat-1Immunoprecipitation1) Collect the cultured cells from 75-cm2 flask (containing about 0. 5-1 x 10e7 cells). 2) Wash the cells 2 times with PBS and suspend with 400 µL of cold Lysis buffer (50 mMHEPES-KOH, pH 7. 5, 250 mM NaCl, 0. 1% NP-40, 5 mM EDTA,10% glycerol) containingappropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, thensonicate briefly (up to 10 seconds). 3) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant toanother tube. 4) Add 50 µL of 50% protein G agarose beads in the supernatant. Incubate it at 4°C withrotating for 60 minutes. 5) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C. Supernatant is equally dividedinto another two tube. 6) Add the mouse IgG1 isotype control antibody or anti-Cdh1/Fzr (DCS-266) monoclonalantibody at the amount as suggested in the APPLICATIONS to the supernatant. Vortexbriefly and incubate with gently agitation for 30-120 minutes at 4°C. 7) Add 20 µL of 50% protein G agarose beads into the tube. Mix well and incubate withgentle agitation for 30-60 minutes at 4°C.
Restrictions:	For Research Use only

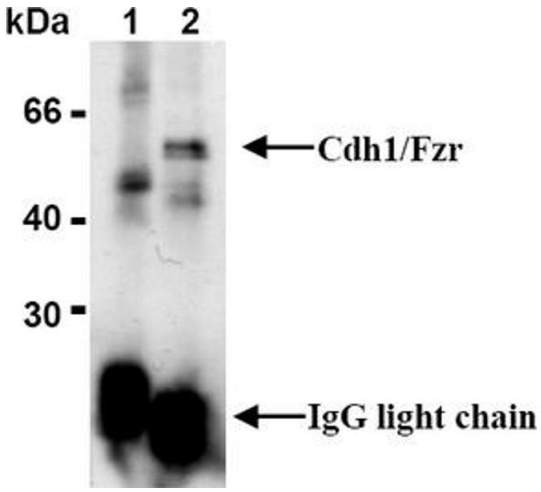
Handling

Concentration:	1.0 mg/mL
Buffer:	PBS, pH 7.2 containing 50 % Glycerol without preservatives.
Preservative:	Without preservative
Storage:	-20 °C

Handling

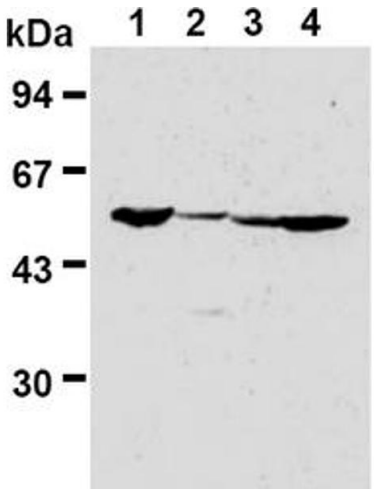
Storage Comment:	Store the antibody undiluted at -20 °C. Shelf life: one year from despatch.
Expiry Date:	12 months

Images



Western Blotting

Image 1.



Western Blotting

Image 2.