Datasheet for ABIN487478

anti-Cyclin D2 antibody

2 Images

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Overview

Quantity:	0.1 mg
Target:	Cyclin D2 (CCND2)
Reactivity:	Human, Mouse
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This Cyclin D2 antibody is un-conjugated
Application:	Western Blotting (WB), Immunoprecipitation (IP)

Product Details

Immunogen:	Full-length recombinant Human Cyclin D2 protein. Remarks: Hybridoma was established by fusion of mouse myeloma cell NS-2 with Balb/cmouse splenocyte.
Clone:	DCS-3
Isotype:	IgG2a
Specificity:	This antibody reacts with Human and Mouse Cyclin D2.
Characteristics:	Synonyms: Cyclin-D2, CCND2
Purification:	Protein-A Sepharose Chromatography.

Target Details

Target:	Cyclin D2 (CCND2)
Alternative Name:	Cyclin D2 (CCND2 Products)

Target Details

Background:	Cyclin D2 (34 kDa) is one of three D-type cyclins that are synthesized in G1 phase and induced in response to agents that promote re-entry into the cell cycle. D-type cyclins assemble with cyclin-dependent kinases Cdk4 and Cdk6 to form complexes that phosphorylate key substrates, including the retinoblastoma protein Rb, involved in proliferation. Generally, D cyclins appear late in the G1 phase of the cell cycle, however, cyclin D2 accumulates in Go and at the G1/S interface. A role for cyclin D2 has been suggested in differentiation and oncogenic transformation. Cyclin D2 is expressed in macrophages in response to CSF-1 stimulation, and overexpression of cyclin D2 is associated with increased in vivo invasiveness of human squamous carcinoma cells.Synonyms: CCND2, Cyclin-D2
Gene ID:	894
UniProt:	P30279
Pathways:	Cell Division Cycle, Mitotic G1-G1/S Phases

Application Details

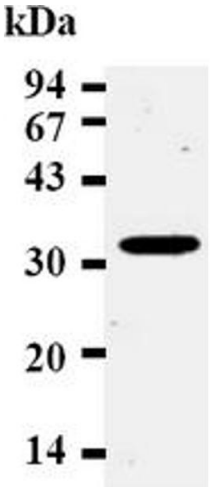
Application Notes:	Western blott: 1 µg/mLImmunoprecipitation: 2 µg/200-300 µL of cell extract. Positive Control: NIH/3T3 cells. It is reported that this clone is reactive with several Human cells such as U-2-OS, Bristol-8and Lovo36 (See Reference 1). Detailed procedure is provided in Protocols. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
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 manufacture's manual for precise transfer procedure. 6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH7. 2) for 1 hour at RT, or overnight at 4°C. 7) Incubate the membrane with the anti-cyclin D2 monoclonal antibody (1 µg/mL) dilutedwith 1% skimmed milk (in PBS, pH 7. 2) for 1 hour at RT8) Wash the membrane with PBS (5 min x 6 times). 9) Incubate the membrane with the 1: 10000POD-conjugated anti-mouse IgG diluted

Application Details

	with 1% skimmed milk (in PBS, pH 7. 2) for 1 h atRT. 10) Wash the membrane with PBS (5 minutes x 6 times)11) Wipe excess buffer on the membrane, then incubate it with appropriatechemiluminescence reagent for 1 minute. Remove extra reagent from the membrane bydabbing with paper towel, and seal it in plastic wrap. 12) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. Thecondition for exposure and development may vary. Positive Control for Western blotting: NIH/3T3Immunoprecipitation1) 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 min at 4°C and transfer the supernatant toanother tube. 3) Add 2 µg of the anti-cyclin D2 monoclonal antibody into 250 µL of the supernatant. Mixwell and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of 50%Protein A-agarose beads resuspended in the Lysis buffer. Mix well and incubate with gentleagitation for 60 min at 4°C. 4) Wash the beads 3-5 times with the ice-cold Lysis buffer (centrifuge the tube at 2,500 x gfor 10 seconds). 5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 min, andcentrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis. (See SDS-PAGE & Western blotting.)
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
Storage Comment:	Store the antibody undiluted at -20 °C. Shelf life: one year from despatch.
Expiry Date:	12 months



Western Blotting

Image 1.

Western Blotting

Image 2.

