Datasheet for ABIN487337

anti-CDC25A antibody

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Overview

Quantity:	0.1 mg
Target:	CDC25A
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This CDC25A antibody is un-conjugated
Application:	Western Blotting (WB), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p)), Immunoprecipitation (IP)

Product Details

Immunogen:	Full-length Human CDC25A fusion protein. Remarks: Hybridoma was established by fusion of Mouse myeloma cell NS-2 with Balb/cmouse splenocyte.
Clone:	DCS-121
Isotype:	IgG2a
Specificity:	This antibody reacts with Human, Mouse and Rat CDC25A.
Cross-Reactivity (Details):	Species reactivity (tested):Human, Mouse and Rat.
Characteristics:	Synonyms: CDC-25A, M-phase inducer phosphatase 1, Dual specificity phosphatase Cdc25A, CellDivision Cycle 25A
Purification:	Protein-A Sepharose Chromatography.

Target Details

Target:	CDC25A
Alternative Name:	CDC25A (CDC25A Products)
Background:	The members of the CDC25 family, CDC25A, CDC25B, and CDC25C, activate the cyclin-dependent kinases at different points in the cell cycle by dephosphorylating key proteins. The ~65 kDa CDC25A protein is a tyrosine phosphatase that regulates the G1/S transition by activating cyclin E/Cdk2 and cyclin A/Cdk2 complexes, which are required for DNA synthesis. CDC25A acts as a checkpoint to prevent DNA replication following DNA damage. DNA damage induces phosphorylation of CDC25A, resulting in its rapid degradation via the ubiquitin-proteosome pathway, and thus silencing Cdk2 activity.Synonyms: CDC-25A, Cell Division Cycle 25A, Dual specificity phosphatase Cdc25A, M-phase inducer phosphatase 1
Gene ID:	993
UniProt:	P30304
Pathways:	Cell Division Cycle, Mitotic G1-G1/S Phases, M Phase

Application Details

Application Notes:	Western Blot: 1-5 µg/mLImmunoprecipitation: 3 µg/200-300 µL of cell extract. Immunohistochemistry: 1-5 µg/mLHeat treatment is necessary for Paraffin Embedded Sections. Microwave oven: 2 times for 10 minutes each in citrate buffer (pH 6.5). Positive Controls: HeLa, Raji, NIH/3T3 and Rat-1 Cells. 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 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

Application Details

	at 4°C. 7) Incubate the membrane with the anti-CDCC25A (DCS-121) monoclonal antibody (1-5µg/mL) diluted with 1% skimmed milk (in PBS, pH 7. 2) for 1 hour at room temperature. 8) Wash the membrane with PBS (5 minutes x 6 times). 9) Incubate the membrane with the 1: 10000 HRP-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) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. Theconditions for exposure and development may vary. Positive Controls for Western blotting: HeLa, Raji, NIH/3T3, Rat-1. Immunoprecipitation1) 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. 3) Add 3 µg of the anti-CDC25A (DCS-121) monoclonal antibody into 250 µL of thesupernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20µL of 50% Protein A-agarose beads resuspended in the Lysis buffer. Mix well and incubatewith gentle agitation for 60 minutes at 4°C. 4) Wash the beads 3-5 times with ice-cold Lysis buffer (centrifuge the tube at 2,500 x g for10 seconds). 5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, andcentrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis. (See SDS-PAGE & Western blotting.)Positive Controls for immunoprecipitation: Raji cells. Immunohistochemical Staining for Paraffin-Embedded Sections: SAB method1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
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

Immunohistochemistry

Image 1.

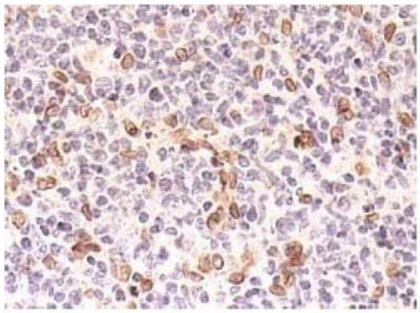

Figure 1.

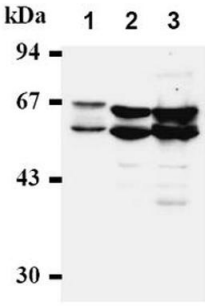

Figure 2.

Western Blotting

Image 2.