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Overview

Quantity:	0.1 mg
Target:	CDKN2C
Reactivity:	Human
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This CDKN2C antibody is un-conjugated
Application:	Western Blotting (WB), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p)), Immunoprecipitation (IP)

Product Details

Immunogen:	Bacterially produced His-tagged p18 proteins.
Clone:	DCS-118
Isotype:	lgG2a
Specificity:	This antibody reacts with Human p18INK4c.
Cross-Reactivity (Details):	Species reactivity (tested):Human.
Characteristics:	Synonyms: p18-INK4c, p18-INK6, p18INK6, CDKN6, Cyclin-dependent kinase 4 inhibitor C,Cyclin-dependent kinase 6 inhibitor
Purification:	Protein-A Sepharose Chromatography.

Target Details

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Target:	CDKN2C
Alternative Name:	CDKN2C / p18INK4c (CDKN2C Products)
Background:	The INK4 family of proteins consists of four members that block progression from the G1-to-S phase of the cell cycle by inhibiting the activity of Cdk4 and Cdk6. The p18INK4c cyclin-dependent kinase inhibitor is an important regulator of cellular differentiation and cell cycle progression, and it also acts as a potent tumor suppressor. p18INK4c is regulated by the transcription factors E2F1 and SP1 in response to environmental and intracellular signals such as cytokines, oncogenic overload, or cellular senescence. Synonyms: CDKN6, Cyclin-dependent kinase 4 inhibitor C, Cyclin-dependent kinase 6 inhibitor, p18-INK4c, p18-INK6, p18INK6
Gene ID:	1031
UniProt:	P42773
Pathways:	Cell Division Cycle, Mitotic G1-G1/S Phases
Application Details	

Application Details

Application Notes:	Western Blot: 1 μ g/mLPositive Control: Saos-2 Cells. Immunoprecipitation: 3 μ g/200-300 μ L of
	cell extract. Positive Control: Saos-2 Cells. Immunohistochemistry: 1-5 μ g/mLHeat treatment is
	necessary for Paraffin Embedded Sections. Microwave oven: 2 times for 10 minutes each in

citrate buffer (pH 6.5). Positive Control: Tonsil Tissue. 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

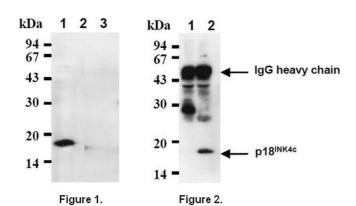
at 4°C. 7) Incubate the membrane with the anti-p18INK4c (DCS-118) monoclonal antibody (1µ g/mL) diluted with 1% skimmed milk (in PBS, pH 7. 2) for 1 hour at room temperature. 8) Wash the membrane with PBS (5 minutes x 6 times). 9) Incubate the membrane with the 1: 10000 HRP-conjugated anti-mouse IgG diluted with 1% 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 appropriate chemiluminescence 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. The conditions for exposure and development may vary. Positive Control for Western blotting: Saos-2 cells. 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) containing appropriate 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 antip18INK4c (DCS-118) monoclonal antibody into 250 µL of the supernatant. 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 for 10 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 Control for immunoprecipitation: Saos-2 cells.

Restrictions:

For Research Use only

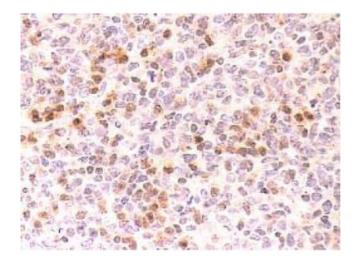
Handling

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.



Immunohistochemistry

Image 2.