OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850
UNITED STATES
Phone: +1-888-267-4436
Fax: +1-301-340-8606
techsupport@origene.com

OriGene Technologies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info-de@origene.com

AM00301AF-N Monoclonal Antibody to CDKN2C / p18INK4c - Azide Free

Alternate names: CDKN6, Cyclin-dependent kinase 4 inhibitor C, Cyclin-dependent kinase 6 inhibitor, p18-INK4c, p18-INK6, p18INK6

Quantity: 0.1 mg

Concentration: 1.0 mg/ml

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.

Uniprot ID: P42773

NCBI: NP_001253

GeneID: 1031

Host / Isotype: Mouse / IgG2a

Clone: DCS-118

Immunogen: Bacterially produced His-tagged p18 proteins.

Format:
State: Liquid purified IgG fraction.
Purification: Protein-A Sepharose Chromatography.
Buffer System: PBS, pH 7.2 containing 50% Glycerol without preservatives.

Applications:
Western Blot: 1 µg/mL
Positive Control: Saos-2 Cells.
Immunoprecipitation: 3 µg/200-300 µL of cell extract.
Positive Control: Saos-2 Cells.
Immunohistochemistry: 1-5 µg/mL
Heat 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.

Specificity: This antibody reacts with Human p18INK4c.

Species Reactivity: **Tested:** Human.

Add. Information: This product was originally produced by MBL International.

Storage: Store the antibody undiluted at -20°C.
Shelf life: one year from despatch.

General Readings:

1. Thullberg, M., et al. Hybridoma 19, 63-72 (2000)
Clone DCS-118 is used in this reference.

Protocols:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-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, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the Lysis buffer 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 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hour in 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, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with the anti-p18INK4c (DCS-118) monoclonal antibody (1 µg/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 by dabbing 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.

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-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, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add 3 µg of the anti-p18INK4c (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 20 µL of 50% Protein A-agarose beads resuspended in the Lysis buffer. Mix well and incubate with 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, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.

(See **SDS-PAGE & Western blotting.)**
Positive Control for immunoprecipitation: Saos-2 cells.

Immunohistochemical Staining for Paraffin-Embedded Sections: SAB method
1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment
Heat treatment by microwave oven: Place the slides put on staining basket in 500 mL beaker with 500 mL citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides 2 times for 10 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.
5) Remove the slides from the citrate buffer and cover each section with 3% H2O2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent for 5 minutes to block non-specific antibody staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with the anti-p18INK4c (DCS-118) monoclonal antibody diluted with PBS containing 1% BSA (1-5 µg/mL).
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody. Incubate for 10 minutes at room temperature. Wash as in step 9.
11) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase. Incubate for 10 minutes at room temperature. Wash as in step 9.
12) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H2O2 in 150 mL PBS. *DAB is a suspected carcinogen and must be handled with care. Always wear gloves.
13) Wash the slides in water for 5 minutes.
14) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
15) Now ready for mounting.
Positive Control for Immunohistochemistry: Tonsil Tissue.

Pictures:

Figure 3. AM00301AF-N p18INK4c antibody Staining of Paraffin Embedded Human Tonsil Section (germinal center).

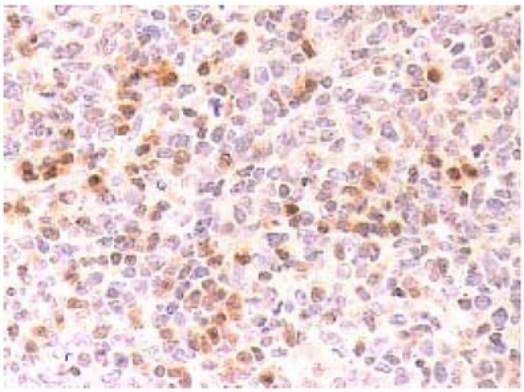

Figure 1. Western blot analysis of p18INK4c expression in Saos-2 cells (lane 1), C2C12 cells (Lane 2) and Rat-1 cells (Lane 3) using p18INK4c antibody (AM00301AF-N). **Figure 2.** Immunoprecipitation of p18INK4c from Saos-2 cells with normal Mouse IgG (Lane 1) or AM00301AF-N (Lane 2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with p18INK4c antibody (AM00301AF-N).

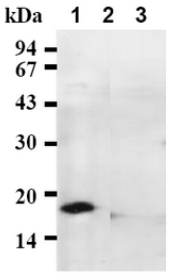

Figure 1.

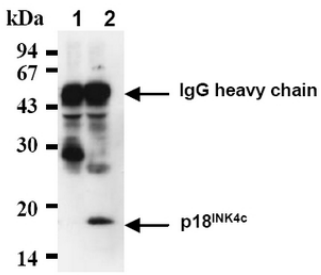

Figure 2.