ProductInformation

ANTI-P16$^{INK4a/CDKN2}$
Developed in Rabbit
IgG Fraction of Antiserum

Product Number **P9981**

Product Description
Anti-p16$^{INK4a/CDKN2}$ is developed in rabbit using synthetic peptides corresponding to amino acid residues 1-16 and 132-148 of human p16$^{INK4a/CDKN2}$ as immunogen. The antibody is purified using protein G.

Anti-p16$^{INK4a/CDKN2}$ recognizes human p16$^{INK4a/CDKN2}$ by immunoblotting (approximately 16 kDa). Other species reactivity is not known. The antibody may also be used for immunoprecipitation.

During the cell cycle of most somatic cells, DNA synthesis (S-phase) and mitosis (M-phase) are separated by two gap phases (G_1 and G_2) of varying duration. Thus, a typical eukaryotic cell sequentially passes through G_1, S, G_2, and M and back into G_1 during a single cycle.[1] Regulation of cell cycle progression in eukaryotic cells depends on the expression of proteins called cyclins.[2] These proteins form complexes with several different cyclin dependent kinases (CDKs). Complexes of cyclins and CDKs play a key role in cell cycle control. Within the complexes, the cyclin subunit serves a regulatory role, whereas the CDKs have a catalytic protein kinase activity.[3] The association of members of the cyclin family with the kinase subunit forms an active kinase, which can initiate M phase of mitosis and meiosis, or function as key regulators of each step of the cell cycle by phosphorylation of several cellular targets. The eukaryotic cell cycle is regulated by the sequential activation of CDKs. The catalytic activity of CDKs is regulated by two general mechanisms, protein phosphorylation and association with regulatory subunits, including the cyclins and the CDK inhibitors (CKIs). Several mammalian CDK inhibitors have been identified which have been divided into two groups on the basis of sequence homology. One group includes p16^{INK4a}, p15^{INK4b}, p18^{INK4c} and p19^{INK4d}, all of which contain characteristic four-fold ankyrin repeats. The second group of CDK inhibitors includes p21^{Cip1}, p27^{Kip1} and p57^{Kip2}. These proteins are structurally and functionally distinct from those of the INK4 family and inhibit CDKs by associating with preactivated cyclin-CDK complexes. p16$^{INK4/CDKN2}$ (also known as p16^{INK4}, p16^{INK4a}, p16^{MTS1}, inhibitor of CDK4), is the product of the *CDKN2* gene. It inhibits the progression of cell cycle through the G_1 phase, by competing with D type cyclins to bind to CDK4 and CDK6. p16$^{INK4/CDKN2}$ is a candidate tumor suppressor, whose gene is frequently deleted or mutated in diverse types of cancer.[4] The availability of monoclonal antibody reacting specifically with p16$^{INK4/CDKN2}$ enables the subcellular detection and localization of p16$^{INK4/CDKN2}$ and the measurement of relative differences in p16$^{INK4/CDKN2}$ levels as a function of cell cycle phase

Reagents
Anti-p16$^{INK4a/CDKN2}$ is supplied as an IgG fraction of antiserum in 0.07 M Tris-glycine, pH 7.4, containing 0.105M NaCl, 30% glycerol and 0.035% sodium azide.

Precautions and Disclaimer
This product contains sodium azide. A material safety data sheet (MSDS) has been sent to the attention of the safety officer at you institution. Consult MSDS for information regarding hazards and safe handling practices.

Storage/Stability
Store at 0 °C to -20 °C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure
Immunoprecipitation
1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1 mg/ml total cell protein in a microcentrifuge tube with PBS (Sigma Product No. P3813).
2. Add 5µg of Anti-p16^{INK4a} to 0.5-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 µl of washed (in PBS) 1:1 slurry of Protein A-Agarose beads (50 µl packed beads) (Sigma Product No. P2545).
5. Gently rock reaction mixture at 4°C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice cold cell lysis buffer (see below) or PBS.
7. Resuspend the agarose beads in 50 µl 2X Laemmli sample buffer.

8. The agarose beads can be frozen or suspended in Laemmli sample buffer and boiled for 5 minutes. Pellet the beads using a microcentrifuge pulse. SDS-PAGE and subsequent immunoblotting analysis may be performed on a sample of the supernatant.

Lysis Buffer:
50 mM Tris-HCl, pH 7.4, containing 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM PMSF, 1 μg/ml each aprotinin, leupeptin, pepstatin, 1 mM Na_3VO_4, and 1 mM NaF.

Product Profile

The recommended dilution is 1μg/ml for immunoblotting using a HeLa nuclear extract. The recommended concentration for immunoprecipitation is 5 μg using human A431 cell lysates.

Note: In order to obtain best results and assay sensitivities of different techniques and preparations, we recommend determining optimal working dilutions by titration test.

References

1. Freeman, R.S., and Donoghue, D.J., Biochemistry, **30**, 2293 (1991).
2. Pines, J., and Hunter, T., J. Cell Biol., **115**, 1 (1991).
3. Yamashita, M., et al., Dev. Growth Differ., **33**, 617 (1991).
4. Aagaard, L., et al., Int. J. Cancer, **61**, 115 (1995).

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