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ProductInformation

Anti-p57 ^{Kip2}
Developed in Rabbit
IgG Fraction of Antiserum

Product Number **P 0357**

Product Description

Anti-p57^{Kip2} is developed in rabbit using a 15 amino acid synthetic peptide ([C]GVGSVEQTPRKRLR) corresponding to amino acids 303-316 of human Kip2 conjugated to KLH as immunogen. The IgG fraction of antiserum is purified using protein A.

Anti-p57^{Kip2} specifically recognizes Kip2. It reacts with bovine and human Kip2 and is expected to cross-react with mouse. Other species reactivity is unknown. The antibody may be used for Immunoblotting (approximately 57 kDa) and 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). These proteins form complexes with several different cyclin dependent kinases (CDKs). Within the complexes, the cyclin subunit serves a regulatory role, whereas the CDKs have a catalytic protein kinase activity.[3] Complexes of cyclins and CDKs play a key role in cell cycle. The eukaryotic cell cycle is regulated by the sequential activation of CDKs. 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 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). Two families of CKIs have been identified. The p21$^{WAF1/Cip1}$ family contains p21$^{WAF1/Cip1}$, p27^{Kip1} and p57^{Kip2} and inhibits all kinases involved in the G1/S transition, whereas the p16^{INK4a}, family, including p15 ^{INK4b}, p16^{INK4a}, p18 ^{INK4c} and p19 ^{INK4d}, inhibit Cdk4 and Cdk6 specifically.[4,5]

The biochemical activities and patterns of expression of CKIs during development, togetherwith data derived from in vitro differentiation systems, implicate these proteins as the primary effectors od signaling pathways that control cell cycle exit, an event that is critical for differentiation. Sudies have shown that p57^{Kip2} (57 kDa, also designated Kip2 57) binds tightly to the G_1 and S phase kinases, cyclin E/Cdk2, cyclin D2/Cdk4, and cyclin A/Cdk2, and to lesser extent to cyclin B/Cdc2, and effectively inhibits their activity.[6] In mammalian cells, p57^{Kip2} localizes to the nucleus, associates with G_1 Cdk components, and its overexpression causes a complete cell cycle arrest in G_1 phase. In contrast to the widespread expression of p21$^{WAF/Cip1}$ and p27^{Kip1} in human tissues, p57^{Kip2} is expressed in a tissue-specific manner,[6] and is not regulated by p53.[7]

Reagents

Anti-p57^{Kip2} is supplied as IgG fraction of antiserum in 0.1 M Tris-glycine, pH 7.4, and 0.15 M sodium chloride containing 0.05% sodium azide.

Protein concentration is approximately 1 mg/ml.

Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) has been sent to the attention of the safety officer at your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

Store at –20 °C. Freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in “frost-free” freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Procedure

Immunoprecipitation

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1 µg/µl total cell protein in a microcentrifuge tube with PBS (Product No. P 3813).
2. Add 5 µg of Anti-p57 ^{Kip2} (P 0357) to 500 µg-1 mg 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) (Product No. P 2545).
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 2× Laemmli sample buffer.
8. The agarose beads can be frozen for later use 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

For immunoblotting, a minimum working antibody concentration 2 µg/ml is recommended using a HeLa nuclear extract and a bovine brain nuclear extract, anti-rabbit IgG-peroxidase conjugate, and a chemiluminescent detection system.

For immunoprecipitation, 5 µg of the antibody is recommended to immunoprecipitate Kip2 from 0.5-1 mg of a HeLa nuclear extract and a bovine brain nuclear extract.

Note: In order to obtain the best results and assay sensitivity in various techniques and preparations, we recommend determining optimal working dilutions by titration.

References

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