

Product Information

Anti-p21^{WAF1/Cip1} Antibody, Mouse Monoclonal

Clone CP74, purified from hybridoma cell culture

P1484

Product Description

Monoclonal Anti-p21^{WAF1/Cip1} (mouse IgG2b isotype) is derived from the CP74 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with recombinant human p21^{WAF1/Cip1}.¹ The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Cat. No. ISO2.

Monoclonal Anti-p21^{WAF1/Cip1} reacts specifically with p21^{WAF1/Cip1}. It shows no cross-reactivity with other closely related mitotic inhibitors. The epitope recognized by the antibody resides in the N terminal amino acids 17-30 within the cyclin-kinase binding domain of the p21^{WAF1/Cip1} molecule.¹ The antibody may be used for ELISA,¹ immunoblotting¹ (21 kDa) and immunoprecipitation (p21/cyclin A/Cdk2 complexes,¹ native and denatured). Reactivity has been observed with human and mouse p21^{WAF1/Cip1}.

During the cell cycle of most somatic cells, DNA synthesis (S-phase) and mitosis (M-phase) are separated by two gap phases (G1 and G2) of varying duration. Thus, a typical eukaryotic cell sequentially passes through G1, S, G2, and M and back into G1 during a single cycle.² Regulation of cell cycle progression in eukaryotic cells depends on the expression of proteins called cyclins.³ 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.⁴ Complexes of cyclins and CDKs play a key role in cell cycle control. 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, which include 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}. These inhibit all kinases involved in the G1/S transition. The p16^{INK4a} family, which includes p15^{INK4b}, p16^{INK4a}, p18^{INK4c} and p19^{INK4d}, inhibits Cdk4 and Cdk6 specifically.⁵

The biochemical activities and patterns of expression of CKIs during development, together with data derived from in vitro differentiation systems, implicate these proteins as the primary effectors of signaling pathways that control cell cycle exit, an event that is critical for differentiation. Studies have shown that p21^{WAF1/Cip1} (21 kDa, also designated Cip1, WAF1, Sdi1, Pic1, CAP20) binds tightly to the G1 and S phase kinases, cyclin E/Cdk2, cyclin D/Cdk4, and cyclin A/Cdk2, and effectively inhibits their activity, whereas p21^{WAF1/Cip1} is relatively a poor inhibitor of the G2/M phase kinase cyclin B/Cdc2.⁶ In addition, p21^{WAF1/Cip1} inhibits proliferating cell nuclear antigen (PCNA), binds to and inhibits inactivation of Rb which is essential for cell cycle progression,⁷ and may also interact with E2F and protect cells against p53-mediated apoptosis.⁸ Indeed, in response to DNA damage, p53 activates the WAF1/CIP1 gene.⁹ p21^{WAF1/Cip1} is also induced in response to TGFβ and during differentiation.¹⁰

The availability of a monoclonal antibody reacting specifically with p21^{WAF1/Cip1} enables the subcellular detection and localization of p21^{WAF1/Cip1} and the measurement of relative differences in p21^{WAF1/Cip1} levels as a function of cell cycle phase.

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide.

Antibody Concentration: ~1 mg/mL.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, or 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.

Product Profile

Immunoblotting: a working concentration of 5-10 µg/mL is determined by using LNCap cells (human prostate adenocarcinoma) treated with MG132 (proteasome inhibition).

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

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