

Product Information

Monoclonal Anti-p27^{Kip1}

Clone DCS-72

Mouse Ascites Fluid

Product Number **P 2092**

Product Description

Monoclonal Anti-p27^{Kip1} (mouse IgG1 isotype) is derived from the DCS-72 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with recombinant p27^{Kip1} protein of rodent origin. The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-p27^{Kip1} recognizes p27^{Kip1} using immunoblotting (27 kDa), immunocytochemistry and immunoprecipitation. Cross-reactivity has been observed with human, monkey, dog, rat and mouse p27^{Kip1}. Higher and lower molecular weight bands, which may represent a polyubiquitinated species and breakdown products of p27^{Kip1}, respectively, may also be detected in some preparations, using immunoblotting.

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.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 ankysin 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. p27, the product of the *kipp1* gene, is an inducible inhibitor of cyclin dependent kinase activity^{4,5}. p27^{Kip1} interacts strongly with D-type cyclins complexed with CDK4 and more weakly with cyclin E and CDK2 complexes. Regulation by p27^{Kip1} may be an essential step in the pathway that links mitogenic signals to cell cycle progression and may be a key molecular event in the physiological process of cell cycle commitment or passage through the restriction point. Thus, the amount of p27^{Kip1} increases in quiescent cells and rapidly decreases after stimulation with specific mitogens. Moreover, constitutive expression of p27^{Kip1} in cultured cells causes cell cycle arrest in G₁. Unlike p21^{Cip1}, the level of p27^{Kip1} in contact-inhibited or serum-deprived cells is relatively high, only to decline after cells emerge from quiescence. The amino-terminal end of the p27^{Kip1} is 42% identical to the p21^{Cip1} amino-terminal end. The availability of monoclonal antibody reacting specifically with p27^{Kip1} enables the subcellular detection and localization of p27^{Kip1} and the measurement of relative differences in p27^{Kip1} levels as a function of cell cycle phase.

Monoclonal Anti-p27^{Kip1} may be used for the localization of p27^{Kip1}, using various immunochemical assays such as immunoblotting, immunocytochemistry, and immunoprecipitation.

Reagents

The product is provided as ascites fluid with 0.1% sodium azide as a preservative.

Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous 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 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.

Product Profile

A minimum working dilution of 1:200 is determined by immunoblotting using a cultured mouse fibroblast line (3T3) extract.

In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

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. Polyak, K., et al., *Cell*, **78**, 59 (1994).
5. Toyoshima, H., and Hunter, T., *Cell*, **78**, 67 (1994).

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