

Product Information

MONOCLONAL ANTI-CDC27

CLONE AF3.1

Purified Mouse Immunoglobulin

Product Number **C 7104**

Product Description

Monoclonal Anti-Cdc27 (mouse IgG2b isotype) is derived from the AF3.1 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with a synthetic peptide corresponding to C-terminal 10 residues (a.a. 814-823) of human Cdc27, conjugated to KLH.¹ The isotype is determined using Sigma ImmunoType™ Kit (Sigma ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma ISO-2).

Monoclonal Anti-Cdc27 reacts specifically with Cdc27. The epitope recognized by the antibody resides within amino acids 814-823 of human Cdc27.¹ The antibody may be used for immunoblotting (97 kDa),^{1,2} immunoprecipitation (immunoprecipitates active APC),¹ immunocytochemistry (4% paraformaldehyde fixation, Triton X-100 permeabilization),² immunohistochemistry (formalin-fixed, paraffin-embedded, and frozen sections),² ELISA¹ and immunoaffinity purification.¹ Cross-reactivity has been observed with human,^{1,2} bovine,² dog,² rat,² mouse² and *Xenopus*.¹

Regulation of cell cycle progression in eukaryotic cells depends on the expression of proteins called cyclins. Mitotic cyclins are subject to stage-specific degradation by ubiquitin-dependent pathways. Ubiquitin is added to proteins by a biochemical "bucket brigade" which passes ubiquitin from an enzyme called E1 to a second enzyme called E2, and finally, aided by a third enzyme called E3, to the target protein.³ Cdc16, Cdc27 and Cdc34 (Cdc16/27/34 complex), which are components of the APC (anaphase-promoting complex), are directly involved as components of the E3 complex.⁴

The Cdc16/27/34 complex functions as a ubiquitin-conjugating enzyme, ubiquitinating cyclin B, and resulting in cyclin B/Cdk complex degradation.⁵ APC is activated at metaphase-anaphase transition and remains active until late G1 phase.⁶

APC activity is regulated by at least four distinct mechanisms, including an activation by Cdc20/p55CDC/Fizzy (Cdc20) and Cdh1/Hct1/Fizzy-related (Cdh1), in a substrate-specific manner.^{2,7-9} Cdc20 and Cdh1 ensure that different substrates of the APC are degraded at the right time during M and G1 phases. Cdc20 targets APC substrates whose degradation is required for the metaphase-anaphase transition (such as Pds1) for degradation, whereas Cdh1 may trigger destruction of substrates whose degradation is important for exit from M phase (such as mitotic cyclins and Ase1).⁷ The timing of APC activation is regulated by the phosphorylation of Cdh1 and Cdc20, by Cdc2-cyclin B (MPF).¹⁰ The regulation of Cdc20 proteolysis reveals a role for the APC components Cdc23 and Cdc27 (97 kDa) during S phase and early mitosis.^{11,12} Monoclonal antibody reacting specifically with Cdc27 is an essential tool in defining the interactions and distributions of Cdc27, its localization to the centrosome and mitotic spindle and its function in the regulation of cell cycle.

Reagent

Monoclonal Anti-Cdc27 is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody Concentration: Approx. 2 mg/ml.

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 °C to 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. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

A working concentration of 1-2 µg/ml is determined by immunoblotting using a HeLa cell nuclear extract.

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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