

Product Information

Anti-cdk-1 (p34^{cdc2})

Developed in Rabbit, Affinity Isolated Antibody

Product Number **C5098**

Product Description

Anti-cdk1 or p34^{cdc2} is developed in rabbit using a synthetic peptide sequence of amino acid residues 264-288 of the C-terminal of mouse cdk1 coupled to KLH as immunogen. The antibody is purified using immunoaffinity chromatography.

Anti-cdk1 recognizes mouse cdk1 (p34^{cdc2}) kinase (34 kD). The antibody may cross-react with other cdc2-like kinases. This antibody also shows species cross reactivity with human, rat and sheep.

This antibody can be used for immunoblotting, immunohistology and immunoprecipitation. It does not block the kinase activity in the immunoprecipitate.

During the cell cycle of most somatic cells, DNA synthesis (S-phase) and mitosis (M-phase) are separated by two "growth" stages (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 Maturation-promoting factor (MPF), originally found during meiosis in frog oocytes, is a cytoplasmic factor which is highly conserved among a wide range of species and plays a key role in the progression of the cell cycle from interphase (G₂) to metaphase (M), in both meiosis and mitosis. One of the components of MPF is a 34 kD protein with kinase activity which is encoded in the fission yeast, *S. pombe*, by the *cdc2* gene (p34^{cdc2}).² The kinase activity and substrate specificity of p34^{cdc2} (also known as Cdk1) change during the cell cycle. These changes have been correlated with both the phosphorylation state of p34^{cdc2} and its association with other proteins called cyclins. Complexes of 'cyclins' and Cdk1 (p34^{cdc2}) play a key role in cell cycle control. Within the complexes, the cyclin subunit serves a regulatory role,³ whereas Cdk1 has a catalytic protein kinase activity.³ Members of the cyclin family of proteins combine with Cdk1 kinase subunit to form active cdc2 kinase, which initiates M phase of mitosis and meiosis. Deactivation of Cdk1 is required for exit from mitosis. Besides involvement in the G₂ to M transition, these complexes function as key regulators of each step of the cell cycle: Cdk1 acts as a catalytic

subunit of MPF when it forms a complex with cyclin B.^{3,4} However, when Cdk1 combines with other types of cyclins, termed G₁ cyclins, it commits the cell to DNA replication. Therefore, the cell cycle can be considered as a Cdk1 cycle which is controlled by biochemical modifications such as phosphorylation of Cdk1 and formation of complex(es) with other proteins, including the cyclins.⁵ In every eukaryote examined, Cdk1 contains an evolutionary conserved 16 amino acid sequence called PSTAIR (EGVPSTAIRESLLKE) which distinguishes Cdk1 from other protein kinases. Nevertheless, other cyclin-dependent kinases, like Cdk2 and Cdk3, contain the PSTAIR motif. The PSTAIR region of Cdk1 is involved in the complex formation with cyclin B.

Cdk1 (p34^{cdc2}) shares 62% homology in protein sequence with p36^{cdc28} protein kinase. In evolutionarily divergent fission and budding yeasts, *cdc2+* and *Cdc28* genes are capable of cross complementation in temperature sensitive mutants. The activity of cdk1 is regulated by association with two distinct peptides, cyclins A or B and p13^{SUC1}. The molecular model of cdk1 is divided into a small and a large lobe with cleft in between. The small lobe is associated with nucleotide binding, the cyclin binding site and the conserved PSTAIRE sequence. A cluster of acidic residues (E38, E40, E41, E42) in the small lobe immediately preceding the PSTAIRE sequence is apparently important for cyclin binding. The large lobe is associated with peptide binding, catalysis and contains two non-contiguous p13^{SUC1} binding sites. p13^{SUC1} is not a substrate for cdk1 but is clearly a critical regulatory component of the cdk1 complex. Mutants that can bind cyclins but have lost the ability to bind p13^{SUC1} are non-functional in *S. pombe*. Cyclin binding precedes and is necessary for the phosphorylation of Thr-161, one of two major phosphorylation sites located on the large lobe at the edge of the cleft. Thr-161 phosphorylation is essential for cdk1 activation, perhaps through stabilization of the cyclin complex. In contrast, phosphorylation of Y15 within the ATP binding site by the Wee1 protein kinase inactivates cdk1 and dephosphorylation by *cdc25* protein phosphatase activates cdk1.

Reagents

The product is supplied as affinity isolated antibody in 0.07 M Tris-glycine buffer, pH 7.0, containing 30% glycerol.

Protein concentration is approximately 0.5 mg/ml by Extinction (E1%, 280nm) = 14.0.

Storage/Stability

Store at 0°C to 120°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 1:1 total cell protein in a microcentrifuge tube with PBS (Sigma Product No. P3813).
2. Add 4 µg of Anti-cdk1 (p34^{cdc2}) to 500 µg - 1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 µl of a 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 or PBS.
7. Resuspend the agarose beads in 50 µl 2X Laemmli sample buffer. The agarose beads can be frozen for later use.
8. Suspend the agarose beads in Laemmli sample buffer and boil 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 Na₃VO₄, and 1 mM NaF.

Immunocytochemistry

1. Plate approximately 200 µl of a cell suspension into each well of a slide. Incubate 24 hours in a 37°C CO₂ incubator.
2. Wash the cells 3 X for 5 min. with PBS. Do not shake cells.

3. Add fixative (95% ethanol, 5% acetic acid) for 1 min. at room temperature.
4. Wash the cells with PBS, 2 X for 15 min. Do not shake cells.
5. Add 400 µl PBS containing 8% BSA and incubate 30 min. at room temperature.
6. Wash cells with PBS for 15 min.
7. Incubate the cells with 10 µg/ml of Anti-cdk1 (p34^{cdc2}) in PBS containing 1% BSA and incubate 1 hr. at room temperature, or overnight at 4°C.
8. Wash the cells 2 X with PBS for 5 min.
9. Incubate the cells with a 1:100 dilution of anti-rabbit IgG conjugated with FITC (Sigma Product No. F9887) in PBS for 1 hr. at room temperature.
10. Wash the cells 3 X with PBS for 5 min.
11. Examine the cells under a fluorescent microscope.

Product Profile

For Immunoblotting, a working concentration of 1 µg/ml will detect cdc2 (~34 kD) from EGF-stimulated human carcinoma A431 or mouse 3T3 fibroblast cell lysates. A second cross-reacting protein (p52) of unknown function is detected in the A431 cells.

For immunocytochemistry, the recommended concentration is 10 µg/ml for staining human carcinoma A431 cells fixed with ethanol:acetic acid (95:5).

For immunoprecipitation, typically 4 µg of the antibody will precipitate cdk1 from human A431 or mouse 3T3 cell lysate containing 0.5 to 1 mg total protein. This antibody does not interfere with kinase activity.

References

- Atherton-Fessler, S., et al., *Mol. Cell Biol.*, **13**, 1675 (1993).
- Brizuela, L., et al., *EMBO J.* **6**(11): 3507, 1987.
- Draetta, G. et al., *Cell* **50**(2): 319, 1987.
- Ducommun B., et al., *Mol. And Cell. Biology* **11**(12): 6177, 1991.
- Freeman, R., and Donoghue, D., *Biochemistry*, **30**, 2293 (1991).
- Hindley, J., and Phear, G.A., *Gene*, **31**, 129 (1984).
- Hirai, T., et al., *Mol. Reprod. Dev.*, **33**, 131 (1992).
- Marcotte M.J., et al., *Mol. And Cell. Biology* **13**(8): 5122, 1993.
- Nurburg, C., and Nurse, P., *Ann. Rev. Biochem.*, **61**, 441 (1992).
- Rattner J.B., et al., *Cell Motility and Cytoskeleton* **17**: 227, 1990.
- Samiei M., et al., *J. Biol. Chem.* **266**: 14889, 1991
- Yamashita, M., et al., *Dev. Growth Differ.*, **33**, 617 (1991).

LPG/RYP 4/99

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.