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ProductInformation

ANTI-CYCLIN E
Developed in Rabbit,
Affinity Isolated Antibody

Product Number **C 4976**

Product Description

Anti-Cyclin E is developed in rabbit using a synthetic peptide (KRKANVTVFLQDPDEEMAKIDRTARDQC) corresponding to amino acids 15-42 of human cyclin E conjugated to KLH as immunogen. The antibody is affinity purified using protein A and peptide resin.

Anti-Cyclin E specifically recognizes human cyclin E (52 kDa by immunoblotting. There is no cross reactivity with other human cyclins. The antibody reacts with human, mouse and rat cyclin E.

Anti-Cyclin E may be used for immunoblotting, immunoprecipitation and immunocytochemistry.

Progression through G1 phase and transition from G1 to S phase of the cell division cycle is controlled by activation of a distinct series of serine/threonine kinase complexes which comprise of a cyclin regulatory subunit and a cyclin dependent kinase (cdk) catalytic subunit. The active cyclin-cdk complexes modify critical target proteins by phosphorylation, thus promoting cell cycle progression towards DNA replication. At least five G1-S phase cyclins have been identified: C, D_1, D_2, D_3, and E. D and E type cyclins govern the rate of progression of mammalian cells through the first gap phase (G1) of the cell cycle and enforced the commitment of cells to replicate their DNA. Cyclin is rate limiting for the G1/S transition. Over expression of Cyclin E accelerates the G1/S transition, decreases cell size and reduces serum requirements, but the overall generation time is unchanged since S and G2 phase become longer. Cyclin E is localized in the nucleus, and forms complexes with Cdk2 (and possibly Cdk3). These complexes have the ability to associate with pRb, to phosphorylate it and hence to accelerate the exit from G1 to S phase.[1]

Reagents

Anti-Cyclin E is affinity isolated and supplied in 0.2 M Tris-glycine, pH 7.4 containing 0.15 M NaCl, 5 mg/ml BSA and 0.05% sodium azide.

Precautions and Disclaimer

Due to the sodium azide content a material safety data 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. Working dilution samples should be discarded if not used within 12 hours.

Procedures

Immunoprecipitation

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1 mg/ml total cell protein in a microcentrifuge tube with PBS (Product No. P 3813).
2. Add 2 µg of anti-Cyclin E 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) (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 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 Na_3VO_4, 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. CO_2 incubator.
2. Wash the cells 3 X for 5 min. with PBS. Do not shake cells.
3. Add fixative (50% ethanol, 50% acetic acid) for 1 min. at room temperature.
4. Wash the cells with PBS, 2 X for 15 min. with gentle agitation. Do not shake cells.
5. Add 400 µl PBS containing 8% BSA and incubate 30 minutes at room temperature.
6. Wash cells with PBS for 15 min.
7. Incubate the cells with 10 µg/ml of anti-Cyclin E in PBS containing 1% BSA and incubate overnight at 4 °C.
8. Wash the cells 2 X with PBS for 15 min.
9. Incubate the cells with a 1:150 dilution of anti-rabbit IgG conjugated with FITC (Product No. F 9887) in PBS for 1.5 hr. at room temperature in the dark.
10. Wash the cells 3 X with PBS for 15 min. in the dark.
11. Mount coverslips with gel mount and allow gel mount to dry in the dark.
12. Examine the cells under a fluorescent microscope.

Product Profile

A working concentration of 0.5-2 µg/ml is recommended for immunoblotting using human A431 and mouse 3T3 RIPA cell lysates.

For immunoprecipitation, 2 µg is recommended to immunoprecipitate cyclin E from 0.5 mg of a human A431 cell lysate.

A working concentration of 10 µg/ml is recommended for immunocytochemistry using human A431 cells fixed with ethanol:acetic acid [50:50].

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

References

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lpg 01/03