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ProductInformation

Anti-phospho-Retinoblastoma (Rb) [pThr^{356}]
Developed in Rabbit, Affinity Isolated Antibody

Product Number **R 3528**

Product Description

Anti-phospho-Retinoblastoma (Rb) [pThr^{356}] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human Rb that contains threonine 356 as immunogen (peptide based on Swiss Protein database, accession number P06400). The sequence is conserved in human, mouse (80% homology) and rat (87 % homology). The antiserum is preadsorbed to remove any reactivity with non-phosphorylated Rb protein. The final product is generated using epitope-specific affinity chromatography.

The antibody detects human Rb protein phosphorylated at threonine 356. Mouse and rat have not been tested. The antibody has been used in immunoblotting applications.

Retinoblastoma protein (Rb), the tumor suppressor product of the retinoblastoma susceptibility gene, is a 110 kDa protein that functions as a negative regulator of the cell cycle. Rb halts inappropriate proliferation by arresting cells in the G1 phase of the cell cycle. At the transcriptional level, Rb protein exerts its growth suppressive function by binding to transcription factors including E2F-1, PU.1, ATF-2, UBF, Elf-1 and c-Abl.[1]

Loss of Rb function leads to uncontrolled cell growth and tumor development and is found in all retinoblastomas and in a variety of other human malignancies including cancers of the breast, lung, colon, prostate, osteosarcomas, soft tissue sarcomas, and leukemia. The ability of Rb protein to alter transcription is regulated by phosphorylation, which is catalyzed by the cyclin-dependent protein kinases (cdks). Rb contains at least 16 consensus sequences for cdk phosphorylation, but the significance of all of these sites is unclear. The dephosphorylation of the Rb protein returns Rb to its active, growth suppressive state.[2-5]

Phosphorylation of Rb on threonine 356 is catalyzed by the complex Cyclin D-cdk4, and plays a role in mediating growth suppression activity of Rb.[6-7]

Reagent

Anti-phospho- Rb [pThr^{356}] is provided in phosphate buffer, pH 7.4 with 1 mg/mL BSA (protease and IgG-free) 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 hazards and safe handling practices.

Storage/Stability

Store at –70 °C. Upon initial thawing freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 12 months when stored appropriately.

Product Profile

The supplied antibody is sufficient for 10 immunoblots.

A recommended working concentration of 0.25 to 0.75 µg/ml is determined by immunoblotting using Jurkat cells in high growth phase.

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

Results

Peptide Competition

The specificity of this phosphorylation site specific antibody was demonstrated by peptide competition experiment using control peptides, which have the sequences of the phosphorylated peptide use as immunogen and the corresponding non-phosphorylated peptide.

1. Extracts prepared from Jurkat cells in high growth phase were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF.
2. Membranes were pre-incubated with following peptides:
   - Lane 1 no peptide
   - Lane 2 a generic peptide containing serine
   - Lane 3 the non-phosphorylated peptide corresponding to the immunogen
   - Lane 4 immunogen
3. Subsequently all four membranes were incubated with 0.50 µg/mL Rb [pThr^{356}] antibody,
4. After washing, membranes were incubated with a conjugate of goat F(ab')$_2$ anti-rabbit IgG and alkaline phosphatase and the bands were visualized.

The data in Figure 1show that only the peptide corresponding to Rb [pThr^{356}] (band 4) blocks the antibody signal, thereby demonstrating the specificity of the antibody.

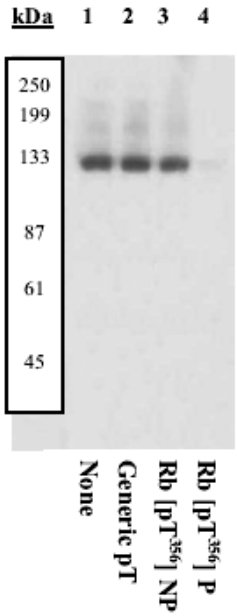

Figure 1 Peptide Competition

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