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Product Information

Anti-phospho-Retinoblastoma (pThr^{821})
produced in rabbit, affinity isolated antibody

Catalog Number **R4153**

Synonym: Anti-phospho-Rb (pThr^{821})

Product Description

Anti-phospho-Retinoblastoma (pThr^{821}) is developed in rabbit using as immunogen a synthetic phosphorylated peptide derived from the region of human Rb (Gene ID# 5925) that contains Thr^{821} (based on Swiss Protein database, accession number P06400). The sequence is conserved in human, mouse, and rat (100% homology). The antiserum is preadsorbed using non-phosphorylated peptide to remove any antibody that reacts with non-phosphorylated Rb protein. The final product is generated using epitope-specific affinity chromatography.

The antibody detects human Rb protein phosphorylated at Thr^{821}. Mouse and rat (100% homology) have not been tested but are expected to react. The antibody has been used in immunoblotting applications.[1-3]

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 G_1 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.

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.[4-7]

Phosphorylation of Thr^{821} is catalyzed by cdk2 complexes such as Cyclin E-cdk2 and Cyclin A-cdk2. It has been demonstrated that phosphorylation of Thr^{821} disrupts Rb interaction with proteins containing the sequence LXCXE. Phosphorylation of this site as well as serine 780, 807, and 811 also disrupts binding to E2F.[1,2,8,9]

Reagent

Supplied in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, with 50% glycerol, 1.0 mg/mL BSA (IgG and protease free), and 0.05% sodium azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at –20 °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.

Immunoblotting: a recommended working concentration of 0.25-1.0 µg/mL is determined by using Jurkat cells in high growth phase.

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

Results

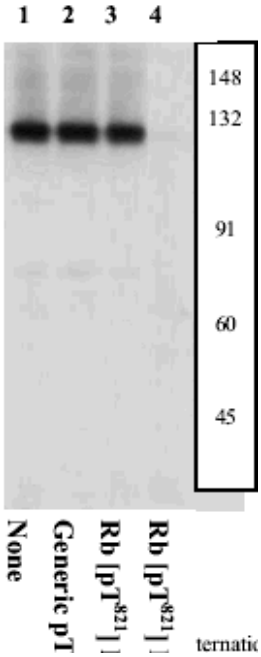
Peptide Competition

The specificity of this phosphorylation site specific antibody, was demonstrated by peptide competition experiment using control peptides.

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 as following:
   - Lane 1 no peptide
   - Lane 2 a generic peptide containing phosphorylated threonine
   - 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 ($pThr^{821}$) 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.

Figure 1

Peptide Competition



The data show that only the peptide corresponding to Rb ($pThr^{821}$) (band 4) blocks the antibody signal, demonstrating the specificity of the antibody.

References

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