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ProductInformation

Anti-phospho-Retinoblastoma (Rb) [pSer^{811}]
Developed in Rabbit, Affinity Isolated Antibody

Product Number **R 4278**

Product Description

Anti-phospho-Retinoblastoma (Rb) [pSer^{811}] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human Rb that contains serine 811 as immunogen (based on Swiss Protein database, accession number P06400). The sequence is conserved in mouse, rat (100% homology) and chicken (82% 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 serine 811. Mouse and rat Rb have not been tested, however at 100% homology they are expected to cross react. 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 cell 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.

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 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.

Phosphorylation of serine 811 is catalyzed by cdk2 complexes such as Cyclin E-cdk2 and Cyclin A-cdk2. Phosphorylation of serines 807 and 811 disrupts binding to c-Abl.

Reagent

Anti-phospho- Rb [pSer^{811}] is provided as a solution in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, with 1.0 mg/ml BSA (IgG and protease 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 reagent is sufficient for 10 blots.

A recommended working concentration of 0.50 to 1.5 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.

1. Extracts prepared from Jurkat cells in high growth phase were resolved by SDS-PAGE on a polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with 5% BSA-Tris buffered saline overnight at 4 °C.

3. Membranes were pre-incubated with the following peptides:
   Lane1 no peptide
   Lane 2 a generic peptide containing phosphorylated serine
   Lane 3 the non-phosphorylated peptide corresponding to the phosphorylated immunogen
   Lane 4 the immunogen
4. Subsequently all four membranes were incubated with 1.0 µg/mL Rb [pSer^{811}] antibody overnight at 4 °C in 3% BSA-TBST buffer.
5. After washing, membranes were incubated with a goat F(ab')$_2$ anti-rabbit IgG alkaline phosphatase conjugate and the bands were visualized.

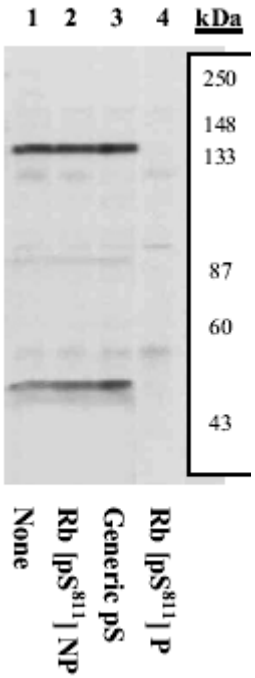

Figure 1 Peptide Competition

The data in Figure 1 show that only the phosphopeptide corresponding to serine 811 blocks the antibody signal, and that the phospho signal is absent after phosphatase stripping. This shows that the Rb [pSer^{811}] antibody produces a phosphospecific signal.

References

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