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ProductInformation

Anti-phospho-Retinoblastoma (Rb) [pSer^{807}]
Developed in Rabbit, Affinity Isolated Antibody

Product Number **R 3778**

Product Description

Anti-phospho-Retinoblastoma (Rb) [pSer^{807}] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human Rb that contains serine 807 as immunogen (based on Swiss Protein database, accession number P06400). The sequence is conserved in human, mouse (100% homology) and rat (92% 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 serine 807. 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 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.[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 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 serine 807 is catalyzed by cdk2 complexes such as Cyclin E-cdk2 and Cyclin A –cdk2. Phosphorylation of serines 612, 780, 807, and 811 disrupts binding to E2F.[6,7]

Reagent

Anti-phospho- Rb [pSer^{807}] is provided in phosphate buffer, pH 7.4 containing 1 mg/ml BSA (protease and IgG-free) and 0.05% sodium azide. The supplied reagent is sufficient for 10 blots.

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

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

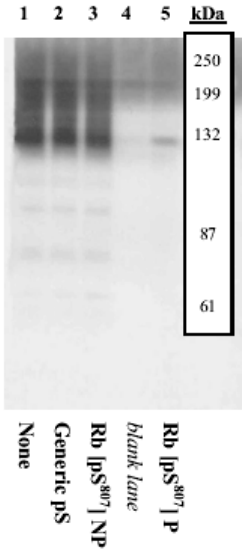
Peptide Competition

The specificity of this a 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 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 left blank to illustrate background
   - Lane 5 immunogen
3. Subsequently all four membranes were incubated with 0.35 µg/mL Rb [pSer^{807}] antibody.
4. After washing, membranes were incubated with a goat F(ab')$_2$ anti-rabbit IgG and alkaline phosphatase conjugate and the bands were visualized.

The data in Figure 1 show that only the peptide corresponding to Rb [pSer^{807}] (Lane 5) blocks the antibody signal, thereby demonstrating the specificity of the antibody.



References

1. Brantley, M.A., Jr. and Harbour, J.W The molecular biology of retinoblastoma. Ocul. Immunol. Inflamm., **9**, 1-8 (2001).
2. Tamrakar, S., et al., Role of pRB dephosphorylation in cell cycle regulation. Front. Biosci., **5**, D121-D137 (2000).
3. Harbour, J.W., et al., Cdk phosphorylation triggers sequential intramolecular interactions that progressively block Rb functions as cells move through G1. Cell, **98**, 859-869 (1999).
4. Driscoll, B., et al., Discovery of a regulatory motif that controls the exposure of specific upstream cyclin-dependent kinase sites that determine both conformation and growth suppressing activity of pRb. J. Biol. Chem., **274**, 9463-9471 (1999).
5. Zarkowska, T. and S. Mittnacht Differential phosphorylation of the retinoblastoma protein by G1/S cyclin-dependent kinases. J. Biol. Chem., 272, 12738-12746 (1997).
6. Knudsen, E.S. and J.Y. Wang Dual mechanisms for the inhibition of E2F binding to RB by cyclin-dependent kinase-mediated RB phosphorylation. Mol. Cell. Biol. **17**, 5771-5783. (1997)
7. Knudsen, E.S. and Wang. J.Y. Differential regulation of retinoblastoma protein function by specific cdk phosphorylation sites. J. Biol. Chem**.**, **271**, 8313-8320 (1996).

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