

## Product Information

### Anti-phospho-Retinoblastoma (Rb) [pSer<sup>807/811</sup>]

Developed in Rabbit, Affinity Isolated Antibody

Product Number **R 3903**

#### Product Description

Anti-phospho-Retinoblastoma (Rb) [pSer<sup>807/811</sup>] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human Rb that contains serines 807 and 811 as immunogen (based on Swiss Protein database, accession number P06400). 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 and mouse Rb protein phosphorylated at serines 807/811. Other species 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.<sup>1</sup>

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.<sup>2-5</sup>

Phosphorylation of serines 807 and 811 is catalyzed by cdk4 complexes such as Cyclin D-cdk4. Recent evidence suggests that phosphorylation of pRb by cdk4 may be required for its subsequent phosphorylation by cdk2. Phosphorylation at serines 612, 780, 807 and 811 disrupts binding to E2F.<sup>6,7</sup>

#### Reagent

Anti-phospho- Rb [pSer<sup>807/811</sup>] is provided in phosphate buffer, pH 7.4 containing 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 reagent is sufficient for 10 blots.

A recommended working concentration of 0.25 to 0.75 µg/ml is determined by immunoblotting using human HeLa, MCF-7 and CEM cells.

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

#### Results

The immunoblot data show that only the peptide corresponding to Rb [p Ser<sup>807/811</sup>] blocks the antibody signal, thereby demonstrating the specificity of the antibody for this phosphorylation site.

#### References

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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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