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Product Information

ANTI-RETINOBLASTOMA PROTEIN (Rb)

Developed in Rabbit
IgG Fraction of Antiserum

Product Number **R 6775**

Product Description

Anti-Retinoblastoma Protein (Rb) is developed in rabbit using a synthetic peptide C-QKMNDSDTSNKEEK corresponding to the C-terminal of human p105 Rb (amino acids 914-928 with N-terminally added cysteine) conjugated to maleimide-activated KLH as immunogen. This sequence is specific for p105 Rb and is not found in p107 Rb or in p130 Rb related proteins. This sequence is highly conserved across species, e.g. with the corresponding rat and mouse p105 Rb sequences. Whole antiserum is fractionated and then further purified by ion-exchange chromatography to provide the IgG fraction of antiserum that is essentially free of other rabbit serum proteins.

Anti-Retinoblastoma Protein recognizes human p105 Rb (105 kDa). Applications include the detection and localization of p105 Rb by immunoblotting and immunohistochemistry. An additional band of 25 kDa may be observed by immunoblotting. Staining of p105 Rb in immunoblotting is specifically inhibited with p105 Rb immunizing peptide (human, amino acids 914-928 with N-terminally added cysteine).

The retinoblastoma (*Rb*) gene product (also known as p105, p110 or pRb) is a tumor suppressor protein that is missing or mutated in a variety of human tumors.¹ In the hereditary childhood form of retinoblastoma, *Rb* is lost from chromosome 13. *Rb* regulates cell proliferation and differentiation by controlling progression through the restriction point within the G₁ phase of the cell cycle.^{1,2} *Rb* forms complexes with many proteins, and this binding activity is required for growth suppression. *Rb* specifically interacts with the E2F family of transcription factors to repress E2F-mediated transcription. *Rb* also interacts and alters the activity of other transcription factors including Elf-1, ATF-2, MyoD, BRG-1, c-myc, N-myc, it interacts with regulatory proteins such as c-Abl tyrosine kinase and with proteins containing a conserved LXCXE motif.¹ *Rb* recruits a histone deacetylase, HDAC1, to E2F-1 to repress transcription.³ Two *Rb* related proteins p107 and p130 also function to regulate specific members of the E2F

transcription factor family. The protein binding function of *Rb* is regulated by a cell cycle-dependent phosphorylation. During G₀/G₁, terminal differentiation and quiescence, *Rb* is underphosphorylated. *Rb* becomes phosphorylated by cyclin-dependent kinases (cdk) as cells progress from G₁ into S phase and throughout the cell cycle. *Rb* contains 16 Ser/Thr-Pro motifs which are potential cdk phosphorylation sites. At least seven of these sites have been shown to be phosphorylated *in-vivo*.⁴ Cell cycle-dependent phosphorylation by cdk2 and cdk4/cdk6 inactivates *Rb* and dissociates *Rb* from E2F transcription factors, thereby activating E2F target genes necessary for cell cycle progression.⁵⁻⁹ *Rb* function is also modulated by the binding of p90MDM2, viral oncoproteins, overexpression of cyclin D and loss of p16^{INK4A}. Induction of the cdk inhibitors p16^{INK4A} and p21^{WAF1} effectively inhibits phosphorylation of the *Rb* protein.

Reagents

Anti-Retinoblastoma Protein (Rb) is supplied as an IgG fraction of antiserum in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

A minimum working dilution of 1:100 is determined by immunoblotting using a whole extract of the human acute lymphoma Jurkat cell line.

A minimum working dilution of 1:5,000 is determined by immunohistochemistry of formalin-fixed, paraffin-embedded sections of human colon adenocarcinoma.

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

References

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