Technical Data Sheet

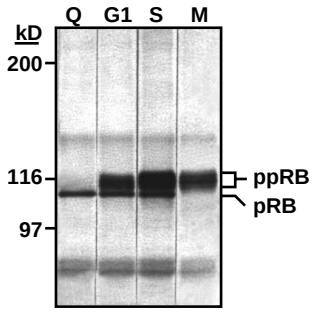
Purified Mouse Anti-Human Retinoblastoma Protein (RB) Monoclonal Antibody

Product Information

Catalog Number:	**554136**
Size:	0.1 mg
Clone:	G3-245
Isotype:	Mouse IgG_1
Storage Buffer:	Aqueous buffered solution containing 0.09% sodium azide.

Background

Members of the retinoblastoma (Rb) family, including the related proteins p107 and p130, share several properties, including the ability to regulate E2F-dependent transcription and to regulate cell-cycle progression. The Rb gene product is a phosphoprotein that is expressed in most normal cells of vertebrates. Rb acts as a tumor suppressor by providing a cell cycle checkpoint between the G1 and S phases *(reviewed in 1-4)*. The active, underphosphorylated form of Rb (Rb or pRb) is primarily found in resting or fully differentiated cells. The activity of Rb is negatively regulated by cyclin-dependent kinases, which phosphorylate Rb in late G1. Thus, the hyperphosphorylated form (ppRb) is primarily found in proliferating cells. pRB inactivation is a critical step leading to S-phase commitment at the G1 checkpoint of the cell cycle. In addition, the underphosphorylated form of Rb may bind to viral oncogenes such as SV40 large T Ag, adenoviral EIA and HPV-E7, which may contribute to the transforming activity of these viral oncoproteins.



Cell cycle expression of retinoblastoma proteins (Rb) in MOLT-4 human leukemia cell line expressing Rb. Rb migrates as multiple bands due to varying degrees of phosphorylation. Whole cell lysates from synchronized MOLT-4 cultures were separated by SDS-PAGE (4-20% gradient). Blots were incubated with anti-Rb (Cat. No. 554136/14001A. Cell cycle stages are denoted as Q (quiescent), G1, S, and M. pRb, underphosphorylated Rb. ppRb, phosphorylated and highly phosphorylated species of Rb.

Specificity and Preparation

G3-245 recognizes an epitope between amino acids 332-344 (DARLFDHDKTLQ) of the human retinoblastoma protein (pp110-114 Rb).[5-8] G3-245 recognizes human,[4,9-12] monkey,[5,13] mouse,[8,14-17] rat,[18] mink and a putative quail Rb.[19] This antibody has also been referred to as Mh-RB-02,20 and mAb-245.[8,17] A Trp-E-Rb fusion protein was used as immunogen.[5] The antibody was purified from ascites or hybridoma tissue culture supernatant by affinity chromatography and is routinely tested by western blot analysis of MOLT-4 human leukemia cells and by immunohistochemical staining of formalin-fixed, paraffin-embedded human tissue sections.

Usage and Storage

Applications include western blot analysis (1-2 µg/ml),[5,6,8,9,11,12,17,20,21,23,25-28] immunoprecipitation (1–2 µg/1x10^6 cells),[5-9,11,23,26] flow cytometry,[11,21] gel shift assays,[1 5] immunofluorescence microscopy of cultured cells (1–5 µg/ml),[18,21,22] and immunohistochemical staining of frozen[7,14,23] and formalin-fixed, paraffin-embedded tissue sections (0.1-1.0 µg/ml).[24] In western blot analysis, Rb migrates as multiple closely-spaced bands between ~110-116 kDa on SDS/PAGE.[1] The different bands represent different Rb phosphorylation states. The level of phosphorylation is cell cycle dependent, and may also be cell type dependent (i.e., not all forms are seen in all cell types that express Rb). Polyacrylamide gel conditions influence the actual number of bands observed. For optimal separation of Rb bands, we recommend a 4-20% gradient gel, </= 12 inches (30 cm) long. MOLT-4 human leukemia cells (ATCC CRL-1582) are suggested as a positive control for western blot analysis. MOLT-4 lysate (Cat. No. 16246Y) is also available as a ready-to-use western blot control. For paraffin-embedded tissue sections, we use standard immunohistochemical staining procedures, including microwave pretreatment with 10 mM citrate buffer prior to antibody staining. Positive Rb staining is typically seen in a variety of human tumors, including subsets of melanomas, colon cancers, stomach cancers, and osteosarcomas. Store the antibody at 4°C.

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Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

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