Technical Data Sheet

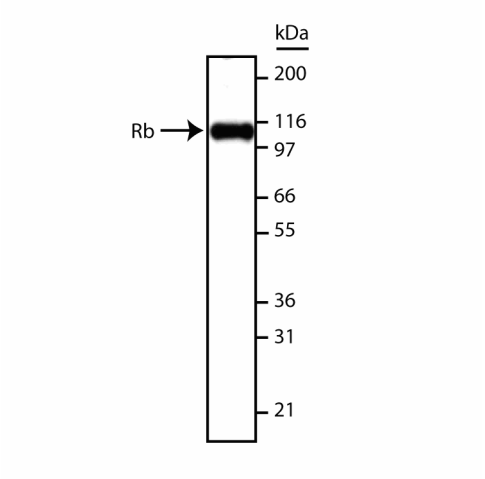
Purified Mouse Anti-Human Retinoblastoma Protein

Product Information

Material Number:	**554140**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	G3-349
Immunogen:	Rb fusion protein
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Target MW:	110-116 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

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. 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 E1A and HPVE7, which may contribute to the transforming activity of these viral oncoproteins. The G3-349 antibody recognizes an epitope located between amino acids 300-380 of human Rb. A truncated Rb fusion protein was used as immunogen.



Western blot analysis of Rb in MOLT-4 human leukemia cell lysate. *The G3-349 antibody (Cat. No. 554140) recognizes phosphorylated and underphosphorylated forms of Rb (~110-116 kDa).*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4° C.

Application Notes

Application

Western blot	Routinely Tested
Immunoprecipitation	Reported

Recommended Assay Procedure:

Applications include immunoprecipitation (1-2 µg/one million cells) and western blot analysis (2 µg/ml). In western blot analysis, Rb forms migrate as multiple closely-spaced bands between ~110-116 kDa on SDS-PAGE. The different bands represent different Rb phosphorylation

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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) can be used as a positive control for western blot analysis.

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. 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.

References

DeCaprio JA, Ludlow JW, Figge J, et al. SV40 large tumor antigen forms a specific complex with the product of the retinoblastoma susceptibility gene. *Cell.* 1988; 54(2):275-283.(Clone-specific: Immunoprecipitation, Western blot)

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