Technical Data Sheet

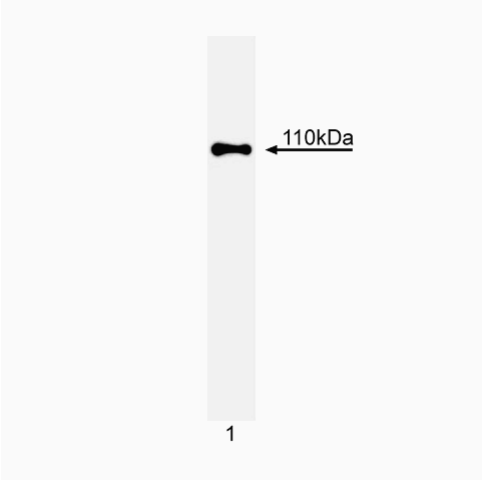
Purified Mouse Anti-Human Rb

Product Information

Material Number:	**554162**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	G99-2005
Immunogen:	Recombinant Human TrpE-Rb
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Target MW:	105-116 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The retinoblastoma gene encodes a nuclear phosphoprotein (Rb or p110[Rb]which is expressed in most normal cells of vertebrates and acts as a tumor suppressor gene product. An underphosphorylated form of Rb is mainly found in resting or fully differentiated cells, whereas the hyperphosphorylated form is present in proliferating cells. Only the underphosphorylated form of Rb binds specifically to viral oncogenes such as SV40 large T, adenoviral EIA and HPV-E7. This interaction may partially contribute to the transforming activity of these viral oncoproteins. Rb also interacts with several cyclins including A, D, and E as well as the transcriptional activator E2F. The importance of these interactions for the biological function of Rb is still being elucidated. The G99-2005 antibody recognizes an epitope in the first 240 amino acids of human Rb. A truncated recombinant human TrpE-Rb fusion protein was used as immunogen.



Western blot analysis of Rb. *Lysate from MOLT-4 human leukemia cells was probed with the G99-2005 antibody). The antibody 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	Tested During Development

Recommended Assay Procedure:

Rb migrates as multiple closely-spaced bands between ~110-116 kDa when sized on denaturing polyacrylamide gels (i.e. by SDS-PAGE). The different bands represent different Rb phosphorylation states, the higher molecular weight bands are more highly phosphorylated than the lower molecular weight bands. The level of phosphorylation is cell cycle dependent, and may also be cell type dependent (not all forms are seen in all cell types that express Rb). Gel conditions influence the actual number of bands observed. In cases where optimal band separation is desired, use a 4 to 20% gradient long (≥12 inches) gel. Applications include immunoprecipitation (1-2 µg/1x10^6 cells) and western blot analysis (2 µg/ml). The antibody is routinely tested by using MOLT-4 human leukemia cells (ATCC CRL-1582).

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Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)

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

Hollingsworth RE Jr, Chen PL, Lee WH. Integration of cell cycle control with transcriptional regulation by the retinoblastoma protein. *Curr Opin Cell Biol Curr Opin Cell Biol.* 1993; 5(2):194-200.(Biology)

Lee WH, Shew JY, Hong FD. The retinoblastoma susceptibility gene encodes a nuclear phosphoprotein associated with DNA binding activity. *Nature.* 1987; 329(6140):642-645.(Immunogen)

Livingston DM. Functional analysis of the retinoblastoma gene product and of RB-SV40 T antigen complexes. *Cancer Surv.* 1992; 12:153-160.(Biology)

Riley DJ, Lee EY, Lee WH. The retinoblastoma protein: more than a tumor suppressor. *Annu Rev Cell Biol.* 1994; 10:1-29.(Clone-specific: Immunoprecipitation, Western blot)