

## Technical Data Sheet

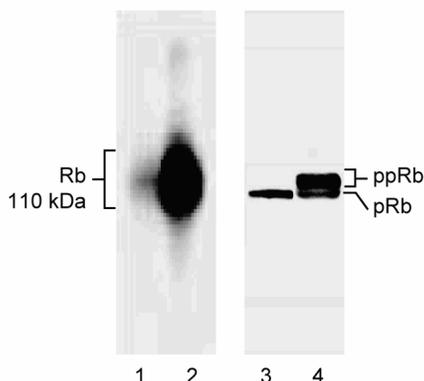
# Purified Mouse Anti-Human Underphosphorylated Retinoblastoma Protein (RB)

## Product Information

Material Number:	554164
Alternate Name:	RB (a.a. 514-610)
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	G99-549
Immunogen:	Recombinant full-length human Rb
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Mouse
Target MW:	105 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

## Description

The retinoblastoma gene encodes a nuclear phosphoprotein (Rb or p110Rb) 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 E1A 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. G99-549 recognizes only the fast migrating, underphosphorylated form of Rb protein. G99-549 cross-reacts with mouse underphosphorylated Rb. The epitope has been mapped to amino acids 514-610 (box A) of human Rb. Recombinant full-length human Rb was produced in the baculovirus expression vector system (BEVS), purified and used as immunogen.



**Selective binding of G99-549 to underphosphorylated Rb.** MOLT-4 cells (ATCC CRL-1582, Human T lymphoblast) were labeled with  $[^{32}P]$ -orthophosphate and immunoprecipitated with either clone G99-549 (lanes 1 & 3) or clone G3-245 (lanes 2 & 4). G99-549 (Cat. No. 554164) recognizes only underphosphorylated Rb whereas G3-245 (Cat. No. 554136) recognizes all species of Rb (highly phosphorylated, phosphorylated and under phosphorylated). **Lanes 1 & 2** - When  $P^{32}$ -labeled IP extracts are run on a gel and detected via autoradiography, the radioactive label is greatly reduced in the G99-549 (lane 1) compared to the G3-245 (lane 2) immunoprecipitates. **Lanes 3 & 4** - A western blot of the same gel probing for total Rb (Clone G3-245) shows that underphosphorylated Rb is present in the G99-549 immunoprecipitates (lane 3) whereas underphosphorylated, phosphorylated, and highly phosphorylated forms are present in the G3-245 immunoprecipitates (lane 4). pRb, underphosphorylated Rb. ppRb, phosphorylated and highly phosphorylated forms of Rb.

## 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

Immunoprecipitation

Routinely Tested

### Recommended Assay Procedure:

Applications include immunoprecipitation at 1-2 µg/one million cells. Rb migrates as multiple, closely-spaced bands between approximately 110-116 kDa when sized on denaturing polyacrylamide gels (i.e. by SDS/PAGE). The multiple 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

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actual number of bands observed. In cases where optimal band separation is desired, use a 7 to 10% non-gradient long (at least 12 inches) gel. MOLT-4 human leukemia cells (ATCC CRL-1582) are suggested as a positive control.

## Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
554136	Purified Mouse Anti-Human Retinoblastoma Protein	0.1 mg	G3-245

## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to [www.bdbiosciences.com/pharming/en/protocols](http://www.bdbiosciences.com/pharming/en/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.
4. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.

## References

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Dunaief JL, Strober BE, Guha S, et al. The retinoblastoma protein and BRG1 form a complex and cooperate to induce cell cycle arrest. *Cell.* 1994; 79(1):119-130. (Clone-specific; Western blot)  
Hollingsworth RE Jr, Chen PL, Lee WH. Integration of cell cycle control with transcriptional regulation by the retinoblastoma protein. *Curr Opin Cell Biol.* 1993; 5(2):194-200. (Biology)  
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. (Biology)  
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