

# Technical Data Sheet

## Purified Mouse Anti-Human Retinoblastoma Protein

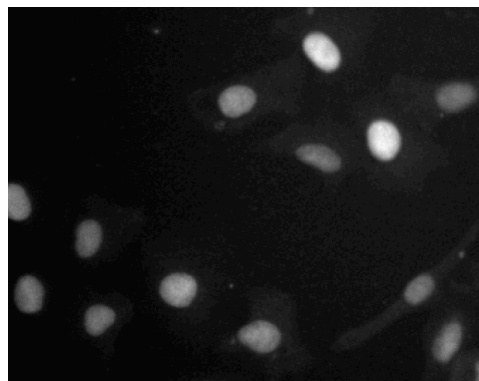
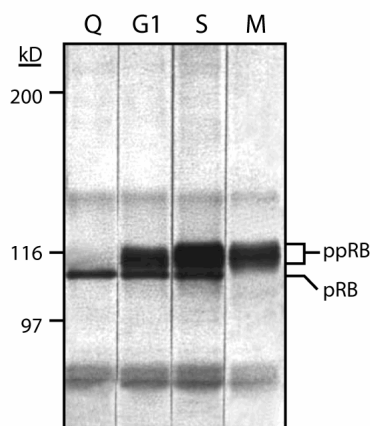
### Product Information

Material Number:	554136
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	G3-245
Immunogen:	Human Rb aa. 332-344
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Reported: Mouse, Rat, Monkey, Quail, Mink
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 oncoproteins such as SV40 large T Ag, adenoviral E1A and HPV-E7, which may contribute to the transforming activity of these viral oncoproteins.

G3-245 was made using a Trp-E-Rb fusion protein as immunogen and recognizes an epitope between amino acids 332-344 (DARLFDHDKTLQ) of the human retinoblastoma protein (pp110-114 Rb). In western blot analysis, Rb migrates as multiple closely-spaced bands between 110-116 kD on SDS-PAGE. The bands represent different Rb phosphorylation states where the level of Rb phosphorylation can be cell cycle and/or cell-type dependent (i.e all forms may not be seen in all cell types that express Rb). G3-245 has been reported to recognize human, monkey, mouse, rat, mink and a putative quail Rb. This antibody has also been referred to as Mh-RB-02,20 and mAb-245.



**Left: 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 at 2 µg/mL (Cat. No. 554136). Cell cycle stages are denoted as Q (quiescent), G1, S, and M. pRb, underphosphorylated Rb. ppRb, phosphorylated and highly phosphorylated species of Rb. **Right: Immunofluorescent staining of A549 (ATCC CCL-185) cells.** Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~10,000 cells per well. After overnight incubation, cells were stained using the alcohol perm protocol and the anti-Rb (332-344) antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). The image was taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained U-2 OS (ATCC HTB-96) and HeLa (ATCC CCL-2) cells using both the Triton™ X-100 and alcohol perm protocols (see Recommended Assay Procedure).

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## Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

## Application Notes

### Application

Intracellular staining (flow cytometry)	Routinely Tested
Western blot	Routinely Tested
Bioimaging	Tested During Development
Immunohistochemistry-formalin (antigen retrieval required)	Reported
Immunohistochemistry-frozen	Reported
Immunoprecipitation	Reported

### Recommended Assay Procedure:

#### Bioimaging

- Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
- Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
- Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
  - Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.OR
  - Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
- Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
- Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
- Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
- Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
- Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
- Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
- Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
- View and analyze the cells on an appropriate imaging instrument.

**Bioimaging:** For more detailed information please refer to [http://www.bdbiosciences.com/support/resources/protocols/certified\\_reagents.jsp](http://www.bdbiosciences.com/support/resources/protocols/certified_reagents.jsp)

**Western blot:** For more detailed information please refer to [http://www.bdbiosciences.com/pharmingen/protocols/Western\\_Blotting.shtml](http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml)

## Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

## Product Notices

- Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
- Triton is a trademark of the Dow Chemical Company.
- 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.
- Please refer to [www.bdbiosciences.com/pharmingen/protocols](http://www.bdbiosciences.com/pharmingen/protocols) for technical protocols.

## References

Bignon YJ, Chen Y, Chang CY, et al. Expression of a retinoblastoma transgene results in dwarf mice. *Genes Dev.* 1993; 7(9):1654-1662. (Clone-specific: Western blot)

Cance WG, Brennan MF, Dudas ME, Huang CM, Cordon-Cardo C. Altered expression of the retinoblastoma gene product in human sarcomas. *N Engl J Med.* 1990; 323(21):1457-1462. (Clone-specific: Immunohistochemistry)

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)

Dowdy SF, Hinds PW, Louie K, Reed SI, Arnold A, Weinberg RA. Physical interaction of the retinoblastoma protein with human D cyclins. *Cell*. 1993; 73(3):499-511. (Clone-specific: Flow cytometry)

Huang S, Lee WH, Lee EY. A cellular protein that competes with SV40 T antigen for binding to the retinoblastoma gene product. *Nature*. 1991; 350(6314):160-162. (Clone-specific: Western blot)

Mittnacht S, Weinberg RA. G1/S phosphorylation of the retinoblastoma protein is associated with an altered affinity for the nuclear compartment. *Cell*. 1991; 65(3):381-393. (Clone-specific: Fluorescence microscopy)

Niwa S, Ueno S, Shirasu R. Alteration of pRb expression in the development of rat tongue carcinoma induced by 4-nitroquinoline 1-oxide. *Oral Oncol*. 2001; 37(7):579-585. (Clone-specific: Immunofluorescence)

Nork TM, Millicchia LL, Poulsen G. Immunolocalization of the retinoblastoma protein in the human eye and in retinoblastoma. *Invest Ophthalmol Vis Sci*. 1994; 35(6):2682-2692. (Clone-specific: Immunohistochemistry)

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