Datasheet for ABIN967405

anti-Retinoblastoma 1 antibody (AA 332-344)

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Overview

Quantity:	0.1 mg
Target:	Retinoblastoma 1 (RB1)
Binding Specificity:	AA 332-344
Reactivity:	Human, Mouse, Rat, Monkey, Mink, Quail
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This Retinoblastoma 1 antibody is un-conjugated
Application:	Western Blotting (WB), Immunoprecipitation (IP), Immunohistochemistry (Frozen Sections) (IHC (fro)), Immunohistochemistry (Formalin-fixed Sections) (IHC (f)), Intracellular Staining (ICS), BioImaging (BI)

Product Details

Brand:	BD Pharmingen™
Immunogen:	Human Rb aa. 332-344
Clone:	G3-245
Isotype:	IgG1
Cross-Reactivity:	Mouse (Murine), Rat (Rattus), Monkey, Quail, Mink
Characteristics:	1. Since applications vary, each investigator should titrate the reagent to obtain optimal results. 2. 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.

Product Details

	3. Triton is a trademark of the Dow Chemical Company. 4. 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. 5. Please refer to us for technical protocols.
Purification:	The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Target Details

Target:	Retinoblastoma 1 (RB1)
Alternative Name:	Rb (RB1 Products)
Background:	Members of the retinoblastoma (Rb) family, including the related proteins p107 and p130, share several properties, including the ability to regulate E2Fdependent 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 EIA 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.
Molecular Weight:	110-116 kDa
Pathways:	Cell Division Cycle, Intracellular Steroid Hormone Receptor Signaling Pathway, Mitotic G1-G1/S Phases, DNA Replication, Maintenance of Protein Location, Synthesis of DNA, Autophagy

Application Details

Application Notes:	Bioimaging 1. Seed the cells in appropriate culture medium at ~10,000 cells per well in an 96-well Imaging Plate and culture overnight. 2. Remove the culture medium from the wells, and fix the cells by adding 100 myl of Fixation Buffer to each well. Incubate for 10 minutes at room temperature (RT). 3. Remove the fixative from the wells, and permeabilize the cells using either 90% methanol, or Triton™ X-100: a. Add 100 myl of -20°C 90% methanol to each well and incubate for 5 minutes at RT. OR b. Add 100 myl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT. 4. Remove the permeabilization buffer, and wash the wells twice with 100 myl of 1× PBS. 5. Remove the PBS, and block the cells by adding 100 myl of to each well. Incubate for 30 minutes at RT. 6. Remove the blocking buffer and add 50 myl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT. 7. Remove the primary antibody, and wash the wells three times with 100 myl of 1× PBS. 8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 myl to each well, and incubate in the dark for 1 hour at RT. 9. Remove the second step reagent, and wash the wells three times with 100 myl of 1× PBS. 10. Remove the PBS, and counter-stain the nuclei by adding 200 myl per well of 2 myg/ml Hoechst 33342 in 1× PBS to each well at least 15 minutes before imaging. 11. View and analyze the cells on an appropriate imaging instrument.
Comment:	Related Products: ABIN967389
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	0.5 mg/mL
Buffer:	Aqueous buffered solution containing ≤0.09 % sodium azide.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	4 °C
Storage Comment:	Store undiluted at 4°C.

Publications

Product cited in:

Niwa, Ueno, Shirasu: "Alteration of pRb expression in the development of rat tongue carcinoma induced by 4-nitroquinoline 1-oxide." in: **Oral oncology**, Vol. 37, Issue 7, pp. 579-85, (2001) (PubMed).

Riley, Lee, Lee: "The retinoblastoma protein: more than a tumor suppressor." in: **Annual review of cell biology**, Vol. 10, pp. 1-29, (1995) (PubMed).

Nork, Millecchia, Poulsen: "Immunolocalization of the retinoblastoma protein in the human eye and in retinoblastoma." in: **Investigative ophthalmology & visual science**, Vol. 35, Issue 6, pp. 2682-92, (1994) (PubMed).

Bignon, Chen, Chang, Riley, Windle, Mellon, Lee: "Expression of a retinoblastoma transgene results in dwarf mice." in: **Genes & development**, Vol. 7, Issue 9, pp. 1654-62, (1993) (PubMed).

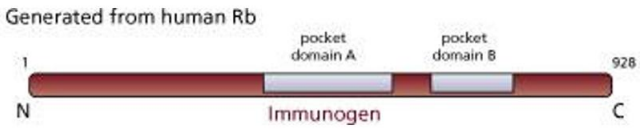
Dowdy, Hinds, Louie, Reed, Arnold, Weinberg: "Physical interaction of the retinoblastoma protein with human D cyclins." in: **Cell**, Vol. 73, Issue 3, pp. 499-511, (1993) (PubMed).

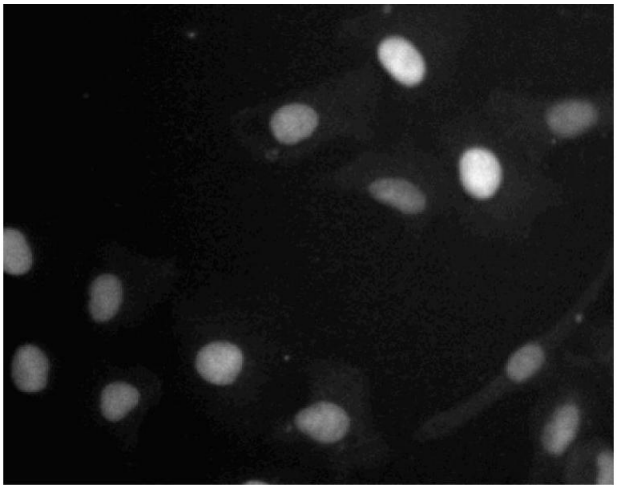
There are more publications referencing this product on: Product page

Images

Western Blotting

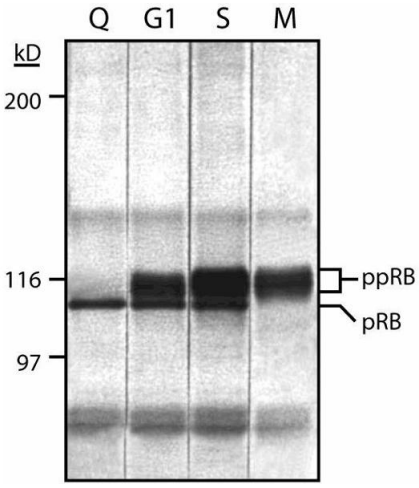
Image 1.





Immunofluorescence

Image 2. Immunofluorescent staining of A549 (ATCC CCL-185) cells. Cells were seeded in a 96 well imaging plate 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. 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.



Western Blotting

Image 3. 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 seperated by SDS-PAGE (4-20% gradient). Blots were incubated with anti-Rb at 2 µg/mL (ABIN967405). Cell cycle stages are denoted as Q (quiescent), G1, S, and M. pRb, underphosphorylated Rb. ppRb, phosphorylated and highly phosphorylated species of Rb.