Technical Data Sheet

Purified Mouse anti-Rb (pS780)

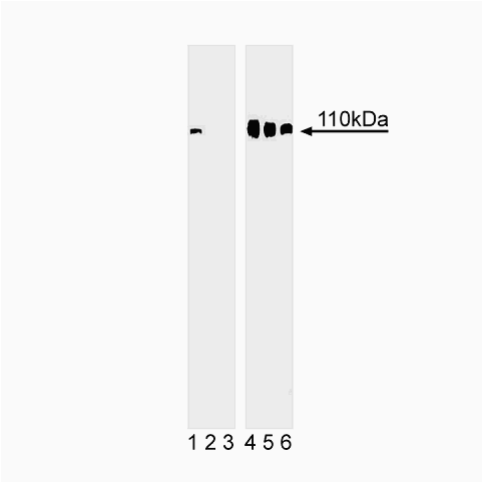
Product Information

Material Number:	**558385**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	J146-35
Immunogen:	Phosphorylated Human Rb
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human
Target MW:	110 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

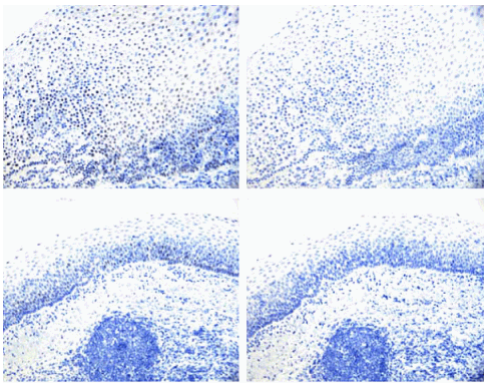
Description

The retinoblastoma gene product (Rb) is well known as a tumor suppressor and is either absent or mutated in many human tumors. Retrovirus-mediated gene transfer of the wild-type Rb gene into several Rb mutant neoplastic cell lines suppresses their tumorgenicity. Rb is a 110-kDa nuclear phosphoprotein that undergoes differential phosphorylation during the cell cycle. During G1 phase, Rb is predominantly in a hypophosphorylated state. It becomes increasingly phosphorylated throughout the cell cycle until late mitosis, when substantial dephosphorylation occurs. Hypophosphorylated Rb interacts with a number of cellular proteins including the E2F transcription factor, several cyclins, RBP-1, RBP-2, c-Abl, c-myc, N-myc, and p46. Phosphorylation of Rb at various sites, by Cyclin-dependent protein kinases, inhibits the binding of Rb to these proteins. Rb is thought to mediate its effects, in part, via the repression of genes required for proliferation. For example, Rb is specifically recruited to promoters containing E2F sites and actively represses E2F mediated transcription. Rb also stimulates the activity of other transcription factors, although the mechanisms are less clearly defined. Thus, Rb appears to regulate transcription in its aim to control cell growth.

The J146-35 monoclonal antibody recognizes Rb phosphorylated at serine 780 (pS780), which affects Rb binding to E2F. The orthologous phosphorylation sites in mouse and rat Rb are serines 773 and 751, respectively.



Western blot analysis of Rb (pS780) in human embryonic skin cells. *Lysates from serum-starved (left panel) and fetal bovine serum-stimulated (right panel) WS1 cell line were probed with purified mouse anti-Rb (pS780) monoclonal antibody at concentrations of 4.0 (lanes 1 and 4), 2.0 (lanes 2 and 5), and 1.0 µg/ml (lanes 3 and 6). Rb (pS780) is identified as a band of 110 kDa in the stimulated cells.*



Rb (pS780) staining on tonsil. *Fresh human tonsil, stimulated with in 5 mM Pervanadate solution for 2 hours (top row) or unstimulated (bottom row), was fixed in formalin and processed. Following antigen retrieval with BD Retrievagen A buffer (Cat. no. 550524), the sections were either left untreated (left column) or treated with a phosphatase to eliminate all phosphorylation (right column). The tissue sections were stained with purified Mouse anti-Rb (pS780) monoclonal antibody with Hematoxylin counterstaining. Original magnification: 20X.*

Preparation and Storage

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

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Application Notes

Application

Western blot	Routinely Tested
Immunohistochemistry-formalin (antigen retrieval required)	Tested During Development

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

Cobrinik D. Pocket proteins and cell cycle control. *Oncogene.* 2005; 24:2796-2809.(Biology)

Knudsen ES, Wang JY. Dual mechanisms for the inihibition of E2F binding to RB by cyclin-dependent kinase-mediate RB phosphorylation. *Mol Cell Biol.* 1997; 17(10):5771-5783.(Biology)