Technical Data Sheet

PE Mouse anti-Rb (pS780)

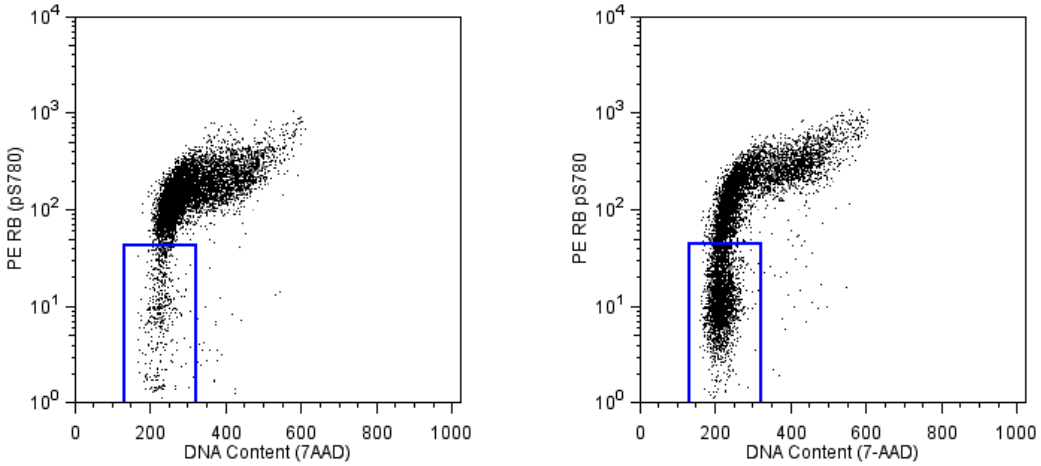
Product Information

Material Number:	**558548**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	J146-35
Immunogen:	Phosphorylated Human Rb Peptide
Isotype:	Mouse (BALB/c) IgG1 κ
Reactivity:	Tested: Human Predicted: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤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.



Analysis of Rb (pS780) in activated human acute lymphoblastic leukemia. *MOLT-4 cells (without serum starvation) were either stimulated with 200 ng/ml PMA (Sigma, Cat. No. P8139) for 24 hours (right panel) or unstimulated (left panel). The cells were fixed (BD™ Phosflow Fix Buffer I, Cat. No. 557870) for 10 minutes at 37°C, then permeabilized (BD™ Phosflow Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes, and then stained with PE Mouse anti-Rb (pS780). 7AAD staining solution (Cat. No. 559925) was used to monitor the cell cycle of the stimulated and unstimulated cells. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system. The boxed region in the data demonstrates that PMA treatment causes cell cycle arrest at the G1 phase, which is associated with dephosphorylation of Rb.*

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Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed by gel filtration chromatography.
Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
558050	Perm Buffer III	125 ml	(none)
557870	Fix Buffer I	250 ml	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 X 10e6 cells in a 100-µl experimental sample (a test).
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/pharmingen/colors.
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

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)