Kit Includes	Quantity	Applications	Reactivity	MW (kDa)	Isotype
Phospho-Rb (Ser780) Antibody #9307	40 µl	W IP E-P	H R Mk	110	Rabbit
Phospho-Rb (Ser795) Antibody #9301	40 µl	W IP	H R Mk	110	Rabbit
Phospho-Rb (Ser807/811) Antibody #9308	40 µl	W IP IHC-P	H R Mk (M)	110	Rabbit
Rb (4H1) Mouse mAb #9309	40 µl	W IP IHC-P IF-IC F ChIP	H Mk B Pg	110	Mouse IgG2a
Anti-rabbit IgG, HRP-linked Antibody #7074	100 µl				Goat
Anti-mouse IgG, HRP-linked Antibody #7076	100 µl				Horse

Applications Key: W=Western Blotting IP=Immunoprecipitation IHC-P=Immunohistochemistry (Paraffin) IF-IC=Immunofluorescence (Immunocytochemistry) F=Flow Cytometry ChIP=Chromatin IP E-P=ELISA (Peptide)

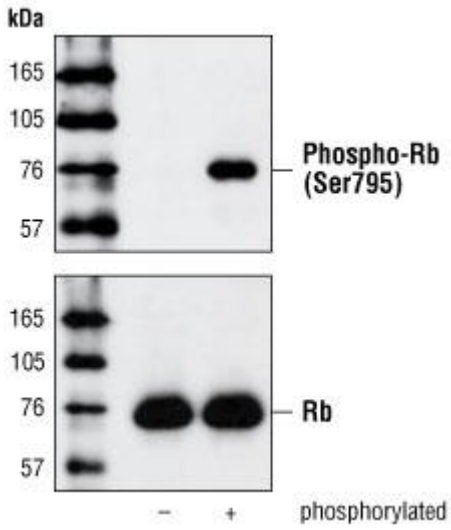
Reactivity Key: H=Human M=Mouse R=Rat Mk=Monkey B=Bovine Pg=Pig

Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Specificity / Sensitivity

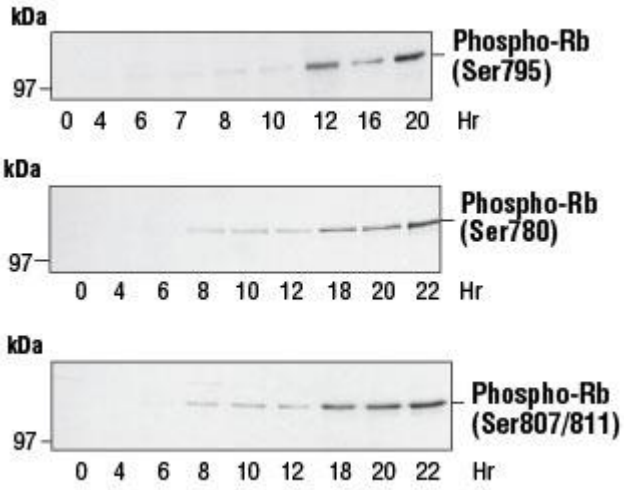
Phospho-Rb (Ser780), Phospho-Rb (Ser795) and Phospho-Rb (Ser807/811) antibodies detect endogenous levels of Rb protein only when phosphorylated at Ser780, Ser795, and Ser807/811, respectively. These antibodies do not cross-react with Rb phosphorylated at these and other sites. Rb (4H1) Mouse mAb detects endogenous levels of total Rb protein. This antibody does not cross-react with the Rb homologues p107, p130 or with other proteins.

Western Blotting



Western blot analysis of Rb Control Protein #9303 using Phospho-Rb (Ser795) Antibody #9301 (upper) or Rb (4H1) mAb #9309 (lower).

Western Blotting



Western blot analysis of Rb phosphorylation in human fibroblasts synchronized by serum deprivation using Phospho-Rb (Ser795) Antibody #9301 (upper), Phospho-Rb (Ser780) Antibody #9307 (middle) and Phospho-Rb (Ser807/811) Antibody #9308 (lower). Cells were synchronized for 24 hours before released by addition of serum and harvested at the times indicated. Cell cycle progression was verified by cyclin analysis and FACS. (Provided by John Boylan, Dupont/Merck, Delaware.)

Description

The Rb Antibody Sampler Kit provides reagents and protocols to investigate cell cycle progression within cells. The kit contains primary and secondary antibodies to perform four Western blot experiments with each antibody.

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues around Ser780, Ser795 and Ser807/811 of human Rb. Antibodies are purified by protein A and peptide affinity chromatography. Rb (4H1) monoclonal antibody is produced by immunizing animals with Rb-C Fusion Protein #6022, which contains residues 701-928 of human Rb .

Background

The retinoblastoma tumor suppressor protein, Rb, regulates cell proliferation by controlling progression through the restriction point within the G1-phase of the cell cycle (1). Rb has three functionally distinct binding domains and interacts with critical regulatory proteins including the E2F family of transcription factors, c-Abl tyrosine kinase, and proteins with a conserved LXCXE motif (2-4). Cell cycle-dependent phosphorylation by a CDK inhibits Rb target binding and allows cell cycle progression (5). Rb inactivation and subsequent cell cycle progression likely requires an initial phosphorylation by cyclin D-CDK4/6 followed by cyclin E-CDK2 phosphorylation (6). Specificity of different CDK/cyclin complexes has been observed *in vitro* (6-8) and cyclin D1 is required for Ser780 phosphorylation *in vivo* (9).

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