Store at –20°C

#9303

Rb Control Proteins

Controls for 10 western blots

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For Research Use Only. Not For Use In Diagnostic Procedures.

Product Includes	Product #	Quantity
Rb Control Proteins (Nonphosphorylated)	26290	100 ul
Rb Control Protein (Phosphorylated)	39812	100 ul

Storage: Supplied in SDS Sample Buffer: 62.5 mM Tris-HCL (ph 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red. Store at -20°C, or at –80°C for long-term storage.

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 CDK's inhibits Rb target binding, thus allowing cell cycle progression (5). Rb inactivation and subsequent cell cycle progression likely requires first 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).

Description: *Nonphosphorylated Rb-C Fusion Protein (5 µg/ml):* Rb-C is expressed as a recombinant fusion protein of Rb residues 701–928 and maltose binding protein serves as a negative control. Supplied in SDS Sample Buffer.

Phosphorylated Rb-C Fusion Protein (5 µg/ml): Rb-C is expressed as a recombinant fusion protein of Rb residues 701–928 and maltose binding protein prepared by in vitro kinase reaction with cdc2 serves as a positive control. Supplied in SDS Sample Buffer.

Note: This truncated Rb recombinant protein is not recognized by Phospho-Rb (Ser608) Antibody #2181 or Rb (D20) Rabbit mAb #9313.

Molecular Weight: Both the nonphosphorylated and phosphorylated forms of Rb-C migrate at an apparent molecular weight of 76 kDa by SDS-PAGE.

Directions for Use: "Boil for 3 minutes prior to use. Load 10 µl of phosphorylated and nonphosphorylated Rb Control Proteins per lane.

Background References:

(1) Sherr, C.J. (1996) *Science* 274, 1672–1677.

(2) Nevins, J.R. et al. (1992) *Science* 258, 424–429.

(3) Welch, P.J. and Wang, J.Y. (1993) *Cell* 75, 779–790.

(4) Hu, Q.J. et al. (1990) *EMBO J.* 9, 1147–1155.

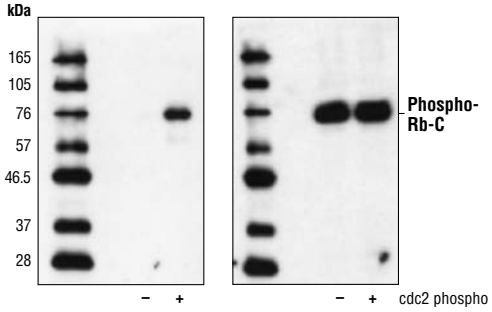
(5) Knudsen, E.S. and Wang, J.Y. (1997) *Mol. Cell. Biol.* 17, 5771–5783.

(6) Lundberg, A.S. and Weinberg, R.A. (1998) *Mol. Cell. Biol.* 18, 753–761.

(7) Connell-Crowley, L. et al. (1997) *Mol. Cell. Biol.* 8, 287–301.

(8) Kitagawa, M. et al. (1996) *EMBO J.* 15, 7060–7069.

(9) Geng, Y. et al. (2001) *Proc. Natl. Acad. Sci. USA* 98, 194–199.



Western blot analysis of Rb-C Fusion Protein #6022 (amino acids 701-928 of Rb fused to MBP) before (-) and after (+) in vitro phosphorylation by cdc2/cyclin B Protein Kinase (New England Biolabs #P6020), using Phospho-Rb (Ser795) Antibody #9301 (left) or the control antibody (right).

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.