#6022 Store at –20°C

Rb-C Fusion Protein

Concentration: 0.5 mg/ml

☐ Small 0.1 mg

☐ Large 0.5 mg

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Description: Rb-C (retinoblastoma protein C-terminus) Fusion Protein serves as a useful substrate for various cyclin-dependent kinases (CDK's) (8,9,11). It is expressed as a recombinant protein fusion of Rb residues 701–928 and maltose binding protein. The phosphorylation sites present in this C-terminal portion of Rb include Ser780 (8), Ser795 (9) and Ser807/811 (11).

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).

Source/Purification: Recombinant protein fusion of Rb residues 701-928 and maltose binding protein.

Quality Assurance: The purified protein was resolved on two identical SDS-polyacrylamide gels. One was stained with Coomassie brilliant blue and the other was blotted to PVDF membrane and the protein band detected using Rb antibody. Greater than 95% of the observable protein was identified as the Rb-C Fusion Protein by apparent molecular weight (68 kDa), and only one band was identified by immunoblotting.

Purification: Purified by the pMAL Protein Purification System (New England Biolabs #E8000S).

Apparent Molecular Weight: 76 kDa

Activity: Rb-C Fusion Protein at a concentration of 2 µg/20 µl reaction can be phosphorylated using cdc2 Protein Kinase (NEB #P6020) in an *in vitro* kinase assay with 1X Kinase Buffer #9802 and 200 µM ATP #9804. After 30 minutes at 30°C, phosphorylation can be detected by Western blot with Phospho-Rb (Ser795) Antibody #9301.

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.

Storage: Supplied in 20 mM Tris-HCl (pH 7.5 at 25°C), 50 mM NaCl, 2 mM Na_2EDTA, 1 mM dithiothreitol (DTT) and 50% glycerol. Store at –20°C.

Companion Products:

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Phospho-Rb (Ser795) Antibody #9301

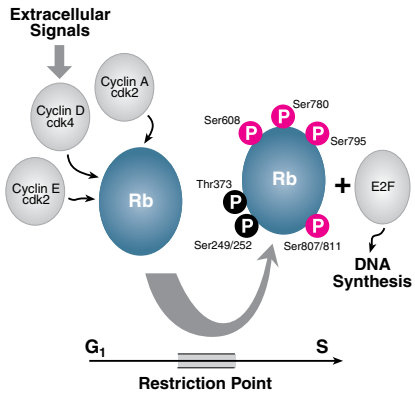
Phospho-Rb (Ser780) Antibody #9307

Phospho-Rb (Ser807/811) Antibody #9308

PhosphoPlus® Rb (Ser780, Ser795, Ser807/811) Antibody Kit #9300

Rb (4H1) Mouse mAb #9309

Rb Antibody Sampler Kit #9969



Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **IC**—Immunocytochemistry **IF**—Immunofluorescence **F**—Flow cytometry **E**—ELISA **D**—DELFIA®

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **X**—Xenopus **Z**—zebra fish **B**—bovine **All**—all species expected

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