

# p-Rb (A-3): sc-377516

## BACKGROUND

Pediatric cancer retinoblastoma and the formation of other human tumors can be attributed to mutations in the retinoblastoma tumor suppressor gene (Rb). The Rb protein regulates differentiation, apoptosis, and cell cycle control by coordinating the cell cycle at G<sub>1</sub>-S with transcriptional machinery. During G<sub>1</sub>, cyclin D-dependent kinase-mediated phosphorylation of Rb at Ser 795 marks the conversion of Rb from a transcriptionally repressive, hypophosphorylated state to an inactive, phosphorylated state, which may be sustained through mitosis by differential phosphorylation of up to 16 putative serine or threonine residues, including Ser 249/Thr 252, Thr 373, Thr 356, Ser 780, Ser 807/Ser 811 and Thr 821/Thr 826. Hypophosphorylated Rb represses the transcription of genes controlling the cell cycle through direct protein-protein interactions and through the recruitment of histone deacetylase.

## REFERENCES

1. Bremner, R., et al. 1995. Direct transcriptional repression by pRB and its reversal by specific cyclins. *Mol. Cell. Biol.* 15: 3256-3265.
2. Weinberg, R.A. 1995. The retinoblastoma protein and cell cycle control. *Cell* 81: 323-330.
3. Sherr, C.J. 1996. Cancer cell cycles. *Science* 274: 1672-1677.
4. Connell-Crowley, L., et al. 1997. Cyclin D1/Cdk4 regulates retinoblastoma protein-mediated cell cycle arrest by site-specific phosphorylation. *Mol. Biol. Cell* 8: 287-301.
5. Luo, R.X., et al. 1998. Rb interacts with histone deacetylase to repress transcription. *Cell* 92: 463-473.
6. Driscoll, B., et al. 1999. Discovery of a regulatory motif that controls the exposure of specific upstream cyclin-dependent kinase sites that determine both conformation and growth suppressing activity of pRb. *J. Biol. Chem.* 274: 9463-9471.
7. Hu, X., et al. 2000. Transforming growth factor beta inhibits the phosphorylation of pRB at multiple serine/threonine sites and differentially regulates the formation of pRB family-E2F complexes in human myeloid leukemia cells. *Biochem. Biophys. Res. Commun.* 276: 930-939.

## CHROMOSOMAL LOCATION

Genetic locus: RB1 (human) mapping to 13q14.2.

## SOURCE

p-Rb (A-3) is a mouse monoclonal antibody epitope corresponding to a short amino acid sequence containing Thr 356 Rb phosphorylated of human origin.

## PRODUCT

Each vial contains 200 µg IgG<sub>2b</sub> in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.

## APPLICATIONS

p-Rb (A-3) is recommended for detection of Thr 356 phosphorylated Rb of human origin by Western Blotting (starting dilution 1:100, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

p-Rb (A-3) is also recommended for detection of correspondingly phosphorylated Rb in additional species, including equine, bovine and porcine.

Suitable for use as control antibody for Rb siRNA (h): sc-29468, Rb shRNA Plasmid (h): sc-29468-SH and Rb shRNA (h) Lentiviral Particles: sc-29468-V.

Molecular Weight (predicted) of p-Rb: 106 kDa.

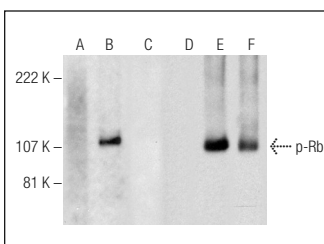
Molecular Weight (observed) of p-Rb: 107-140 kDa.

Positive Controls: SK-LMS-1 cell lysate: sc-3813 or MOLT-4 cell lysate: sc-2233.

## RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent), Western Blotting Luminol Reagent: sc-2048 and Lambda Phosphatase: sc-200312A. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## DATA



Western blot analysis of Rb phosphorylation in non-transfected: sc-117752 (A, D), untreated human Rb transfected: sc-114014 (B, E) and lambda protein phosphatase (sc-200312A) treated human Rb transfected: sc-114014 (C, F) 293T whole cell lysates. Antibodies tested include p-Rb (A-3): sc-377516 (A, B, C) and Rb (M-153): sc-7905 (D, E, F).

## STORAGE

Store at 4° C, \*\*DO NOT FREEZE\*\*. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.