

p-Rb (83.T821/826): sc-135777

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 G₁-S with transcriptional machinery. During G₁, 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. Barrientes, S., et al. 2000. Glutamic acid mutagenesis of retinoblastoma protein phosphorylation sites has diverse effects on function. *Oncogene* 19: 562-570.

CHROMOSOMAL LOCATION

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

SOURCE

p-Rb (83.T821/826) is a mouse monoclonal antibody raised against a short amino acid sequence containing Thr 821 and Thr 826 dually phosphorylated Rb of human origin.

PRODUCT

Each vial contains 200 µg IgG_{2b} kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin. Also available as TransCruz reagent for Gel Supershift and ChIP applications, sc-135777 X, 200 µg/0.1 ml.

STORAGE

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

APPLICATIONS

p-Rb (83.T821/826) is recommended for detection of Thr 821 and Thr 826 dually phosphorylated Rb of human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)] and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

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.

p-Rb (83.T821/826) X TransCruz antibody is recommended for Gel Supershift and ChIP applications.

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

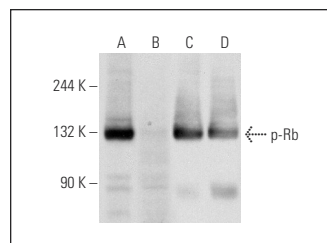
Molecular Weight (observed) of p-Rb: 107-140 kDa.

Positive Controls: Rb (h): 293T Lysate: sc-114014, MOLT-4 cell lysate: sc-2233 or K-562 whole cell lysate: sc-2203.

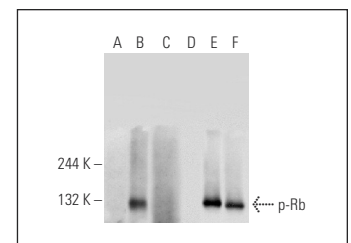
RECOMMENDED SUPPORT REAGENTS

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent), Lambda Phosphatase: sc-200312A and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml).

DATA



Western blot analysis of Rb phosphorylation in untreated (A,C) and lambda protein phosphatase treated (B,D) MOLT-4 whole cell lysates. Antibodies tested include p-Rb (83.T821/826): sc-135774 (A,B) and Rb (M-153): sc-7905 (C,D).



Western blot analysis of Rb phosphorylation in non-transfected (A,D), untreated human Rb transfected (B,E) and lambda protein phosphatase treated human Rb transfected (C,F) 293T whole cell lysates. Antibodies tested include p-Rb (83.T821/826): sc-135777 (A,B,C) and Rb (M-153): sc-7905 (D,E,F).

RESEARCH USE

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

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.