

Rb (h): 293T Lysate: sc-114014

BACKGROUND

Pediatric cancer retinoblastoma and the formation of other human tumors can be attributed to mutations in the retinoblastoma tumor suppressor gene. The retinoblastoma tumor suppressor gene product, known as Rb or p-Rb, regulates differentiation, apoptosis and cell cycle control by coordinating the cell cycle at G₁/S with transcriptional machinery that includes the heterodimeric E2F family. During G₁, cyclin D (D1, D2, D3)-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 cell cycle through direct protein-protein interactions by binding and inactivating the promoters of transcription factors and through recruitment of histone deacetylase, which deacetylates promoter regions and enhances nucleosome formation, thereby masking transcription enhancing *cis* elements.

REFERENCES

- Weinberg, R.A. 1995. The retinoblastoma protein and cell cycle control. *Cell* 81: 323-330.
- Bremner, R., Cohen, B.L., Sopta, M., Hamel, P.A., Ingles, C.J., Gallie, B.L. and Phillips, R.A. 1995. Direct transcriptional repression by p-Rb and its reversal by specific cyclins. *Mol. Cell. Biol.* 15: 3256-3265.
- Sherr, C.J. 1996. Cancer cell cycles. *Science* 274: 1672-1677.
- Connell-Crowley, L., Harper, J.W. and Goodrich, D.W. 1997. Cyclin D1/Cdk4 regulates retinoblastoma protein-mediated cell cycle arrest by site-specific phosphorylation. *Mol. Biol. Cell* 8: 287-301.
- Luo, R.X., Postigo, A.A. and Dean, D.C. 1998. Rb interacts with histone deacetylase to repress transcription. *Cell* 92: 463-473.
- Driscoll, B., T'Ang, A., Hu, Y.H., Yan, C.L., Fu, Y., Luo, Y., Wu, K.J., Wen, S., Shi, X. H., Barsky, L., Weinberg, K., Murphree, A.L. and Fung, Y.K. 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 p-Rb. *J. Biol. Chem.* 274: 9463-9471.
- Hu, X., Cress, W.D., Zhong, Q. and Zuckerman, K.S. 2000. Transforming growth factor β inhibits the phosphorylation of p-Rb at multiple serine/threonine sites and differentially regulates the formation of p-Rb family-E2F complexes in human myeloid leukemia cells. *Biochem. Biophys. Res. Commun.* 276: 930-939.
- Barrientes, S., Cooke, C. and Goodrich, D.W. 2000. Glutamic acid mutagenesis of retinoblastoma protein phosphorylation sites has diverse effects on function. *Oncogene* 19: 562-570.
- LocusLink Report (LocusID: 5925). <http://www.ncbi.nlm.nih.gov/LocusLink/>

STORAGE

Store at -20° C. Repeated freezing and thawing should be minimized. Sample vial should be boiled once prior to use. Non-hazardous. No MSDS required.

CHROMOSOMAL LOCATION

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

PRODUCT

Rb (h): 293T Lysate represents a lysate of human Rb transfected 293T cells and is provided as 100 μ g protein in 200 μ l SDS-PAGE buffer.

APPLICATIONS

Rb (h): 293T Lysate is suitable as a Western Blotting positive control for human reactive Rb antibodies. Recommended use: 10-20 μ l per lane.

Control 293T Lysate: sc-117752 is available as a Western Blotting negative control lysate derived from non-transfected 293T cells.

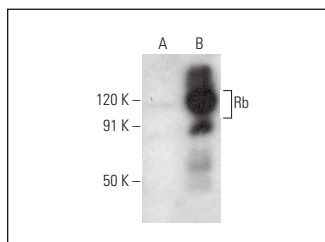
Rb (IF8) HRP: sc-102 HRP is recommended as a positive control antibody for Western Blot analysis of enhanced human Rb expression in Rb transfected 293T cells (starting dilution 1:100, dilution range 1:100-1:1,000).

RECOMMENDED SUPPORT REAGENTS

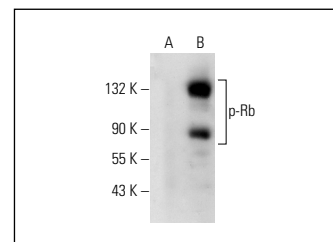
To ensure optimal results, the following support reagents are recommended:

1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048.

DATA



Rb (IF8) HRP: sc-102 HRP. Direct western blot analysis of Rb expression in non-transfected: sc-117752 (A) and human Rb transfected: sc-114014 (B) 293T whole cell lysates.



p-Rb (Tyr356) (7F10): sc-56175. Western blot analysis of phosphorylated Rb expression in non-transfected: sc-117752 (A) and human Rb transfected: sc-114014 (B) 293T whole cell lysates.

RESEARCH USE

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

PROTOCOLS

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