SANTA CRUZ BIOTECHNOLOGY, INC.

p-Rb (B-7): sc-377527

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

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_1-S with transcriptional machinery. During G_1, 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.

CHROMOSOMAL LOCATION

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

SOURCE

p-Rb (B-7) is a mouse monoclonal antibody epitope corresponding to a short amino acid sequence containing Thr 356 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.

p-Rb (B-7) is available conjugated to agarose (sc-377527 AC), 500 µg/0.25 ml agarose in 1 ml, for IP; to HRP (sc-377527 HRP), 200 µg/ml, for WB, IHC(P) and ELISA; to either phycoerythrin (sc-377527 PE), fluorescein (sc-377527 FITC), Alexa Fluor® 488 (sc-377527 AF488), Alexa Fluor® 546 (sc-377527 AF546), Alexa Fluor® 594 (sc-377527 AF594) or Alexa Fluor® 647 (sc-377527 AF647), 200 µg/ml, for WB (RGB), IF, IHC(P) and FCM; and to either Alexa Fluor® 680 (sc-377527 AF680) or Alexa Fluor® 790 (sc-377527 AF790), 200 µg/ml, for Near-Infrared (NIR) WB, IF and FCM.

Blocking peptide available for competition studies, sc-377527 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% stabilizer protein).

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 (B-7) 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, immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500) 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.

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

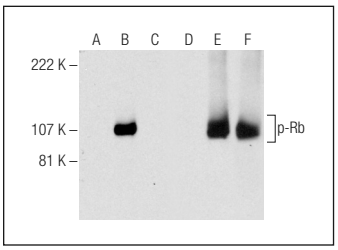
Molecular Weight (observed) of p-Rb: 107-140 kDa.

Positive Controls: Rb (h): 293T Lysate: sc-114014, SK-LMS-1 cell lysate: sc-3813 or MOLT-4 cell lysate: sc-2233.

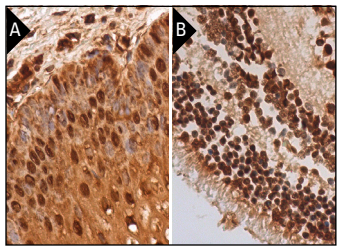
RECOMMENDED SUPPORT REAGENTS

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). 3) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850. 4) Immunohistochemistry: use m-IgGκ BP-HRP: sc-516102 with DAB, 50X: sc-24982 and Immunohistomount: sc-45086, or Organo/Limonene Mount: sc-45087.

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 (B-7): sc-377527 (**A**,**B**,**C**) and Rb (M-153): sc-7905 (**D**,**E**,**F**).



p-Rb (B-7): sc-377527. Immunoperoxidase staining of formalin fixed, paraffin-embedded human vagina tissue showing nuclear and cytoplasmic staining of squamous epithelial cells (**A**). Immunoperoxidase staining of formalin fixed, paraffin-embedded human fetal eye tissue showing nuclear staining of cells in retina (**B**).

RESEARCH USE

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