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SANTA CRUZ BIOTECHNOLOGY, INC.

Rb siRNA (h2): sc-44273

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 pRb, regulates differentiation, apoptosis and cell cycle control by coordinating the cell cycle, at G_1/S, with transcriptional machinery that includes the heterodimeric E2F family. The G_1, 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

1. Weinberg, R.A. 1995. The retinoblastoma protein and cell cycle control. Cell 81: 323-330.
2. Bremner, R., et al. 1995. Direct transcriptional repression by pRb and its reversal by specific cyclins. Mol. Cell. Biol. 15: 3256-3265.
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.

CHROMOSOMAL LOCATION

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

PRODUCT

Rb siRNA (h2) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Rb shRNA Plasmid (h2): sc-44273-SH and Rb shRNA (h2) Lentiviral Particles: sc-44273-V as alternate gene silencing products.

For independent verification of Rb (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44273A, sc-44273B and sc-44273C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNases and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Rb siRNA (h2) is recommended for the inhibition of Rb expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

Rb (IF8): sc-102 is recommended as a control antibody for monitoring of Rb gene expression knockdown by Western blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

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, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Rb gene expression knockdown using RT-PCR Primer: Rb (h2)-PR: sc-44273-PR (20 µl, 554 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.