

P15RS siRNA (h): sc-76024

BACKGROUND

The normal progression of cells through the cell cycle is under the control of the cyclin dependent protein kinases (Cdks), which are subject to inhibition by the mitotic inhibitory INK4 family. p15 is a member of the INK4 family and acts as a cyclin dependent kinase inhibitor to prevent Cdk kinase activation. P15RS (cyclin dependent kinase 2B-inhibitor-related protein), a 213 amino acid protein that contains an RPR domain, is involved in the regulation of nuclear pre-mRNA, which suggests that P15RS acts as a negative regulator of the G₁ phase of the cell cycle. The expression of P15RS is unregulated in cells that overexpress p15, further suggesting a role for P15RS in cell cycle regulation. The gene that encodes P15RS is located on chromosome 18q12.2.

REFERENCES

1. Quesnel, B., et al. 1998. Methylation of the p15^{INK4b} gene in myelodysplastic syndromes is frequent and acquired during disease progression. *Blood* 91: 2985-2990.
2. Staller, P., et al. 2001. Repression of p15^{INK4b} expression by Myc through association with Miz-1. *Nat. Cell Biol.* 3: 392-399.
3. Liu, J., et al. 2002. Identification and characterization of P15RS, a novel P15^{INK4b} related gene on G₁/S progression. *Biochem. Biophys. Res. Commun.* 299: 880-885.
4. Tanaka, T.S., et al. 2002. Gene expression profiling of embryo-derived stem cells reveals candidate genes associated with pluripotency and lineage specificity. *Genome Res.* 12: 1921-1928.
5. Daskalakis, M., et al. 2002. Demethylation of a hypermethylated P15/INK4B gene in patients with myelodysplastic syndrome by 5-Aza-2'-deoxycytidine (decitabine) treatment. *Blood* 100: 2957-2964.

CHROMOSOMAL LOCATION

Genetic locus: RPRD1A (human) mapping to 18q12.2.

PRODUCT

P15RS siRNA (h) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see P15RS shRNA Plasmid (h): sc-76024-SH and P15RS shRNA (h) Lentiviral Particles: sc-76024-V as alternate gene silencing products.

For independent verification of P15RS (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-76024A, sc-76024B and sc-76024C.

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.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

P15RS siRNA (h) is recommended for the inhibition of P15RS 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 μ M in 66 μ l. 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

P15RS (C-6): sc-514724 is recommended as a control antibody for monitoring of P15RS 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-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. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 P15RS gene expression knockdown using RT-PCR Primer: P15RS (h)-PR: sc-76024-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.