

RCK siRNA (m): sc-72247

BACKGROUND

RCK, also known as DDX6 and P54, is a member of the DEAD-box RNA helicase family of proteins, all of which share common protein motifs. Found in most tissues, RCK is an unwindase that exhibits ATP-dependent RNA unwinding activity, as well as the ability to decay RNA in the 5'-3' direction. In non-malignant cells, RCK is associated with all processes of normal RNA metabolism including splicing, export and translation initiation. Mutations in the gene encoding RCK can cause the protein to be overexpressed, changing its function to that of an oncogene that positively regulates the expression of genes involved in cell growth and proliferation. It is believed that, through its unwindase activity, the main function of RCK is to downregulate mRNA expression and maintain normal transcriptional levels within the cell.

REFERENCES

1. Akao, Y., et al. 1995. The RCK/p54 candidate proto-oncogene product is a 54 kDa DEAD box protein differentially expressed in human and mouse tissues. *Cancer Res.* 55: 3444-3449.
2. Akiyama, K., et al. 1999. Expression of two DEAD box genes (DDX1 and DDX6) is independent of that of MYCN in human neuroblastoma cell lines. *Biochem. Mol. Biol. Int.* 47: 563-568.
3. Smillie, D.A. and Sommerville, J. 2002. RNA helicase p54 (DDX6) is a shuttling protein involved in nuclear assembly of stored mRNP particles. *J. Cell Sci.* 115: 395-407.
4. Akao, Y., et al. 2003. A tumour-associated DEAD-box protein, RCK/p54 exhibits RNA unwinding activity toward c-Myc RNAs *in vitro*. *Genes Cells* 8: 671-676.
5. Matsumoto, K., et al. 2005. Expression of RCK/p54, a DEAD-box RNA helicase, in gametogenesis and early embryogenesis of mice. *Dev. Dyn.* 233: 1149-1156.
6. Matsui, T., et al. 2006. Structural insight of human DEAD-box protein RCK/p54 into its substrate recognition with conformational changes. *Genes Cells* 11: 439-452.

CHROMOSOMAL LOCATION

Genetic locus: Ddx6 (mouse) mapping to 9 A5.2.

PRODUCT

RCK siRNA (m) 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 RCK shRNA Plasmid (m): sc-72247-SH and RCK shRNA (m) Lentiviral Particles: sc-72247-V as alternate gene silencing products.

For independent verification of RCK (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-72247A, sc-72247B and sc-72247C.

RESEARCH USE

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

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

RCK siRNA (m) is recommended for the inhibition of RCK expression in mouse 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

RCK (E-12): sc-376433 is recommended as a control antibody for monitoring of RCK 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 RCK gene expression knockdown using RT-PCR Primer: RCK (m)-PR: sc-72247-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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