

RECK siRNA (r): sc-270171

BACKGROUND

RECK (reversion-inducing-cysteine-rich protein with Kazal motifs) is a membrane anchored glycoprotein that binds to and inhibits the proteolytic activity of matrix metalloproteinase-9 (MMP-9). The enzymatic activity of MMP-9 facilitates tumor invasion by proteolytically digesting the extracellular matrix, thereby enabling tumor growth, expansion and metastasis. RECK inhibits the secretion and activation of MMP-9 into the extracellular matrix, which results in the inhibition of tumor growth. RECK contains multiple EGF-like repeats and serine-protease inhibitor-like domains. The expression of RECK is suppressed in several tumors and oncogenically transformed cells, suggesting that the loss of RECK activity correlates with transformed phenotypes. Transcriptional activation of RECK is potentially negatively regulated by the Sp1 family of transcription factors, as it contains two Sp1 binding motifs in the promoter region, and, in cells transformed with the ras oncogene, the Sp1 promoter region is essential for repressing RECK gene expression.

REFERENCES

1. DeClerck, Y.A., et al. 1992. Inhibition of invasion and metastasis in cells transfected with an inhibitor of metalloproteinases. *Cancer Res.* 52: 701-708.
2. Himelstein, B.P., et al. 1997. Transcriptional activation of the matrix metalloproteinase-9 gene in an H-ras and v-myc transformed rat embryo cell line. *Oncogene* 14: 1995-1998.
3. Giambernardi, T.A., et al. 1998. Overview of matrix metalloproteinase expression in cultured human cells. *Matrix Biol.* 16: 483-496.
4. Takahashi, C., et al. 1998. Regulation of matrix metalloproteinase-9 and inhibition of tumor invasion by the membrane-anchored glycoprotein RECK. *Proc. Natl. Acad. Sci. USA* 95: 13221-13226.
5. Sasahara, R.M., et al. 1999. Oncogene-mediated downregulation of RECK, a novel transformation suppressor gene. *Braz. J. Med. Biol. Res.* 32: 891-895.
6. Sasahara, R.M., et al. 1999. Involvement of the Sp1 site in ras-mediated downregulation of the RECK metastasis suppressor gene. *Biochem. Biophys. Res. Commun.* 264: 668-675.

CHROMOSOMAL LOCATION

Genetic locus: Reck (rat) mapping to 5q22.

PRODUCT

RECK siRNA (r) 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 RECK shRNA Plasmid (r): sc-270171-SH and RECK shRNA (r) Lentiviral Particles: sc-270171-V as alternate gene silencing products.

For independent verification of RECK (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270171A, sc-270171B and sc-270171C.

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

RECK siRNA (r) is recommended for the inhibition of RECK expression in rat 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

RECK (G-4): sc-373929 is recommended as a control antibody for monitoring of RECK 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 RECK gene expression knockdown using RT-PCR Primer: RECK (r)-PR: sc-270171-PR (20 μ l). 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.