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Rheb siRNA (m): sc-41860

BACKGROUND

H-, K- and N-Ras represent the prototype members of a family of small G proteins which are frequently activated to an oncogenic state in a wide variety of human tumors. Activation is due to point mutations at position 12 or 61 within their coding sequence. Such mutations cause these proteins to be constitutively converted to their active GTP-bound rather than the inactive GDP-bound state. The related human R-Ras gene was initially cloned by low stringency hybridization methods. Position 38 or 87 mutants of R-Ras (analogous to positions 12 and 61 in H-Ras) have been shown to be capable of activating oncogenic function. Ras p21 in its active GTP binding state binds to Raf-1, resulting in activation of the MAP kinase signaling cascade. An additional member of the Ras family, Rheb (Ras-related GTP-binding protein), also interacts with Raf-1. This interaction is potentiated by growth factors and agents that increase cAMP levels.

REFERENCES

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3. Lowe, D.G., et al. 1987. Heterologous expression and characterization of the human R-ras gene product. *Mol. Cell. Biol.* 7: 2845-2856.
4. Bos, J.L. 1989. Ras oncogenes in human cancer: a review. *Cancer Res.* 49: 4682-4689.
5. Saez, R., et al. 1994. Oncogenic activation of human R-ras by point mutations analogous to those of prototype H-ras oncogenes. *Oncogene* 9: 2977-2982.
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CHROMOSOMAL LOCATION

Genetic locus: Rheb (mouse) mapping to 5 A3.

PRODUCT

Rheb 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 Rheb shRNA Plasmid (m): sc-41860-SH and Rheb shRNA (m) Lentiviral Particles: sc-41860-V as alternate gene silencing products.

For independent verification of Rheb (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-41860A, sc-41860B and sc-41860C.

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

Rheb siRNA (m) is recommended for the inhibition of Rheb 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

Rheb (80-R): sc-130398 is recommended as a control antibody for monitoring of Rheb 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 Rheb gene expression knockdown using RT-PCR Primer: Rheb (m)-PR: sc-41860-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.