

R-Ras siRNA (h): sc-36336

BACKGROUND

H-, K- and N-Ras represent the prototype members of a family of small G proteins that are frequently activated to an oncogenic state in a wide variety of human tumors. Activation is due to point mutations at either position 12 or 61 within their coding sequence. Such mutations cause these proteins to be constitutively converted to their active, rather than the inactive, GDP-bound state. The related human R-Ras gene was initially cloned by low stringency hybridization methods. The R-Ras protein has been shown to interact with the Bcl-2 gene product involved in a signaling pathway that intervenes with apoptosis. Positions 38 and 87 (analogous to positions 12 and 61 in H-Ras) mutants of R-Ras have been shown to be capable of activating oncogenic function. Data has been obtained indicating that R-Ras may exert its biological effect by means of modulating the activity of the Raf-1 kinase on its direct downstream effectors.

REFERENCES

1. Barbacid, M. 1987. Ras genes. *Annu. Rev. Biochem.* 56: 779-827.
2. Lowe, D.G., et al. 1987. Structure of the human and murine R-Ras genes, novel genes closely related to Ras proto-oncogenes. *Cell* 48: 137-146.
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. Fernandez-Sarabia, M.J., et al. 1993. Bcl-2 associates with the Ras-related protein R-Ras p23. *Nature* 366: 274-275.

CHROMOSOMAL LOCATION

Genetic locus: RRAS (human) mapping to 19q13.33.

PRODUCT

R-Ras 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 R-Ras shRNA Plasmid (h): sc-36336-SH and R-Ras shRNA (h) Lentiviral Particles: sc-36336-V as alternate gene silencing products.

For independent verification of R-Ras (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-36336A, sc-36336B and sc-36336C.

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

R-Ras siRNA (h) is recommended for the inhibition of R-Ras 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

R-Ras (C-8): sc-166221 is recommended as a control antibody for monitoring of R-Ras 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 R-Ras gene expression knockdown using RT-PCR Primer: R-Ras (h)-PR: sc-36336-PR (20 μ l, 575 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Titushkin, I., et al. 2011. Altered osteogenic commitment of human mesenchymal stem cells by ERM protein-dependent modulation of cellular biomechanics. *J. Biomech.* 44: 2692-2698.

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.