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Rho A siRNA (h2): sc-44209

BACKGROUND

The Ras p21 family of guanine nucleotide proteins has been widely studied in view of its apparent role in signal transduction pathways and high frequency of mutations in human malignancies. It is now clear, however, that the Ras proteins (H-, K- and N-Ras p21) are members of a much larger superfamily of related proteins. Six members of this family, Rap 1A, Rap 1B, Rap 2, R-Ras, Ral A and Ral B, exhibit approximately 50% amino acid homology to Ras. The six mammalian Rho proteins (Rho A, B, C, G, 7 and 8) are approximately 30% homologous to Ras and are expressed in a wide range of cell types. Both Ras p21 and Rho p21, as well as other members of the Ras superfamily, contain a carboxy-terminal CAAX sequence (C, cysteine; A, aliphatic amino acid; X, any amino acid) which in the case of Ras has been shown to be essential for correct localization and function.

REFERENCES

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4. Chardin, P. 1988. The Ras superfamily proteins. *Biochimie* 70: 865-868.
5. Olofsson, B., et al. 1988. Expression of the Ras-related Ral A, Rho 12 and Rab genes in adult mouse tissues. *Oncogene* 3: 231-234.
6. Morris, J.D., et al. 1989. Scrape-loading of Swiss 3T3 cells with Ras protein rapidly activates protein kinase C in the absence of phospholipid hydrolysis. *Oncogene* 4: 27-31.
7. Garrett, M.D., et al. 1989. Identification of distinct cytoplasmic targets for Ras/R-Ras and Rho regulatory proteins. *J. Biol. Chem.* 264: 10-13.
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CHROMOSOMAL LOCATION

Genetic locus: RHOA (human) mapping to 3p21.31.

PRODUCT

Rho A siRNA (h2) 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 Rho A shRNA Plasmid (h2): sc-44209-SH and Rho A shRNA (h2) Lentiviral Particles: sc-44209-V as alternate gene silencing products.

For independent verification of Rho A (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44209A, sc-44209B and sc-44209C.

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

Rho A siRNA (h2) is recommended for the inhibition of Rho A 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

Rho A (26C4): sc-418 is recommended as a control antibody for monitoring of Rho A 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 Rho A gene expression knockdown using RT-PCR Primer: Rho A (h2)-PR: sc-44209-PR (20 μ l, 488 bp). 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.