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Rho A/B/C shRNA (h) Lentiviral Particles: sc-44076-V

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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2. Yeramian, P., et al. 1987. Nucleotide sequence of human Rho cDNA clone 12. *Nucl. Acids Res.* 15: 189.
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4. 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.
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6. 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.
7. Morris, J.D.M., et al. 1989. Scrape-loading of Swiss 3T3 cells with Ras protein rapidly activates protein kinase C in the absence of phospholipase hydrolysis. *Oncogene* 4: 27-31.
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PRODUCT

Rho A/B/C shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Rho A/B/C siRNA (h): sc-44076 and Rho A/B/C shRNA Plasmid (h): sc-44076-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

Rho A/B/C shRNA (h) Lentiviral Particles is recommended for the inhibition of Rho A, Rho B and Rho C expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

Rho A/B/C (F-1): sc-166399 is recommended as a control antibody for monitoring of Rho A, Rho B and Rho C 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Rho A, Rho B and Rho C gene expression knockdown using RT-PCR Primer: Rho A/B/C (h)-PR: sc-44076-PR (20 μ l). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.