SANTA CRUZ BIOTECHNOLOGY, INC.

Rho D siRNA (h): sc-60032

BACKGROUND

Upon activation, the small GTPase Rho D (also designated RhoHP1 and ARHD) contributes to rearrangement of the actin cytoskeleton and cell surface and also governs endosome motility and distribution. The effects of Rho D antagonize those of its family member, Rho A, by dissasembling actin stress fibers normally enhanced by Rho A. Additionally, Rho D disengages focal adhesions, resulting in retardation of cell migration. Accordingly, transfection of a constitutively active form of Rho D (designated Rho D G26V) reverses the invasive phenotype of $G_{\alpha\ olf}$ induced cells, implying the possiblity of a therapeutic use for activated Rho D in counteracting tumor metastasis.

REFERENCES

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2. Ruiz-Argüelles, G.J., et al. 1993. The infusion of anti-Rho-(D) opsonized erythrocytes may be useful in the treatment of patients, splenectomized or not, with chronic, refractory autoimmune thrombocytopenic purpura—a prospective study. Am. J. Hematol. 43: 72-73.
3. Mohandas, K., et al. 1994. Loss and reappearance of Rho(D) antigen on the red blood cells of an individual with acute myelogenous leukemia. Immunohematology 10: 134-135.
4. Murphy, C., et al. 1996. Endosome dynamics regulated by a Rho protein. Nature 384: 427-432.
5. Shimizu, F., et al. 1997. Isolation of a novel human cDNA (RhoHP1) homologous to Rho genes. Biochim. Biophys. Acta 1351: 13-16.
6. Tsubakimoto, K., et al. 1999. Small GTPase RhoD suppresses cell migration and cytokinesis. Oncogene 18: 2431-2440.
7. Kim, H.S., et al. 2000. Assignment of the human RhoHP1 gene (ARHD) to chromosome 11q14.3 by radiation hybrid mapping. Cytogenet. Cell Genet. 89: 53.
8. Regnauld, K., et al. 2002. G-protein α(olf) subunit promotes cellular invasion, survival, and neuroendocrine differentiation in digestive and urogenital epithelial cells. Oncogene 21: 4020-4031.

CHROMOSOMAL LOCATION

Genetic locus: RHOD (human) mapping to 11q13.2.

PRODUCT

Rho D 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Rho D shRNA Plasmid (h): sc-60032-SH and Rho D shRNA (h) Lentiviral Particles: sc-60032-V as alternate gene silencing products.

For independent verification of Rho D (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-60032A, sc-60032B and sc-60032C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Rho D siRNA (h) is recommended for the inhibition of Rho D 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 µM in 66 µl. 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 D (H-6): sc-365241 is recommended as a control antibody for monitoring of Rho D 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 D gene expression knockdown using RT-PCR Primer: Rho D (h)-PR: sc-60032-PR (20 µl). 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.