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SANTA CRUZ BIOTECHNOLOGY, INC.

Rho GDIγ siRNA (m): sc-41878

BACKGROUND

The Ras superfamily of small GTP-binding proteins are critical mediators of diverse cell signaling pathways, including those leading to proliferation, cytoskeletal organization and secretion. The counter-conversion of the active GTP-bound form of these proteins to their inactive GDP-bound form is influenced by two types of regulatory proteins: those that alter the intrinsic GTPase activity of the GTP-binding proteins and those that alter the rate of GDP/GTP exchange. Guanine nucleotide-releasing factors (GRFs) increase the GDP dissociation rate, while GDP-dissociation inhibitors (GDIs) decrease the dissociation rate. The Rho GDI subfamily is composed of Rho GDIα, Ly-GDI (also known as Rho GDIβ and previously known as GDI/D4) and Rho GDIγ. The Rho GDI proteins interact with and have varying affinities for several Ras-like GTP binding proteins, including Rho A, Rho B, Rac and Cdc42. Ly-GDI is expressed only in hematopoietic cells, predominantly in B and T lymphocyte cell lines.

REFERENCES

1. Trahey, M., et al. 1987. A cytoplasmic protein stimulates normal N-Ras p21 GTPase, but does not affect oncogenic mutants. Science 238: 542-545.
2. Hall, A. 1990. The cellular functions of small GTP-binding proteins. Science 249: 635-640.
3. Bourne, H.R., et al. 1990. The GTPase superfamily: a conserved switch for diverse cell functions. Nature 348: 125-132.
4. Garrett, M.D., et al. 1991. Purification and N-terminal sequence of the p21Rho GTPase-activating protein, Rho GAP. Biochem. J. 276: 833-836.
5. Scherle, P., et al. 1993. Ly-GDI, a GDP-dissociation inhibitor of the RhoA GTP-binding protein, is expressed preferentially in lymphocytes. Proc. Natl. Acad. Sci. USA 90: 7568-7572.
6. Platko, J.V., et al. 1995. A single residue can modify target-binding affinity and activity of the functional domain of the Rho-subfamily GDP dissociation inhibitors. Proc. Natl. Acad. Sci. USA 92: 2974-2978.
7. Adra, C.N., et al. 1997. RhoGDIγ: a GDP-dissociation inhibitor for Rho proteins with preferential expression in brain and pancreas. Proc. Natl. Acad. Sci. USA 94: 4279-4284.

CHROMOSOMAL LOCATION

Genetic locus: Arhgdig (mouse) mapping to 17 A3.3.

PRODUCT

Rho GDIγ 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Rho GDIγ shRNA Plasmid (m): sc-41878-SH and Rho GDIγ shRNA (m) Lentiviral Particles: sc-41878-V as alternate gene silencing products.

For independent verification of Rho GDIγ (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-41878A, sc-41878B and sc-41878C.

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 GDIγ siRNA (m) is recommended for the inhibition of Rho GDIγ 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 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 GDIγ (E-1): sc-393690 is recommended as a control antibody for monitoring of Rho GDIγ 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 GDIγ gene expression knockdown using RT-PCR Primer: Rho GDIγ (m)-PR: sc-41878-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.