

Ly-GDI siRNA (h): sc-35826

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 GDI α , Ly-GDI (also known as Rho GDI β and previously known as GDI/D4) and Rho GDI γ . 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. and McCormick, F. 1987. A cytoplasmic protein stimulates normal N-Ras p21 GTPase, but does not affect oncogenic mutants. *Science* 238: 542-545.
2. Bourne, H.R., et al. 1990. The GTPase superfamily: a conserved switch for diverse cell functions. *Nature* 348: 125-132.
3. Hall, A. 1990. The cellular functions of small GTP-binding proteins. *Science* 249: 635-640.
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 Rho A 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. RhoGDI γ : 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: ARHGDI B (human) mapping to 12p12.3.

PRODUCT

Ly-GDI 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 Ly-GDI shRNA Plasmid (h): sc-35826-SH and Ly-GDI shRNA (h) Lentiviral Particles: sc-35826-V as alternate gene silencing products.

For independent verification of Ly-GDI (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-35826A, sc-35826B and sc-35826C.

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

Ly-GDI siRNA (h) is recommended for the inhibition of Ly-GDI 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

Ly-GDI (G-12): sc-376473 is recommended as a control antibody for monitoring of Ly-GDI 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 Ly-GDI gene expression knockdown using RT-PCR Primer: Ly-GDI (h)-PR: sc-35826-PR (20 μ l, 522 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.

PROTOCOLS

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