

Rhotekin 2 siRNA (h): sc-90523

BACKGROUND

Rho, the Ras-related small GTPase, is responsible for the regulation of Actin-based cytoskeletal structures including stress fibers, focal adhesions and the contractile ring apparatus. Rho proteins function as molecular switches that are able to turn cytokinesis on and off. Although little is known about signaling downstream of Rho, a host of putative Rho effector proteins have been described, including raphilin, Rhotekin, citron and the serine/threonine kinase, protein kinase N. Rhotekin 2, also known as PLEKHK1 (Pleckstrin homology domain-containing family K member 1), is a 609 amino acid protein that is expressed in bone marrow-derived cells, lymphocytes and CD4 positive T-cells. Due to this expression pattern, it is likely that Rhotekin 2 participates in lymphopoiesis. Rhotekin 2 contains one PH domain, a motif that is present in a variety of proteins that are involved in intracellular signaling. There are three isoforms of Rhotekin that are produced as a result of alternative splicing events.

REFERENCES

1. Reid, T., et al. 1996. Rhotekin, a new putative target for Rho bearing homology to a serine/threonine kinase, PKN, and raphilin in the Rho-binding domain. *J. Biol. Chem.* 271: 13556-13560.
2. Collier, F.M., et al. 2004. Identification and characterization of a lymphocytic Rho-GTPase effector: Rhotekin-2. *Biochem. Biophys. Res. Commun.* 324: 1360-1369.
3. Chen, D., et al. 2004. Bone morphogenetic proteins. *Growth Factors* 22: 233-241.
4. Gregorio-King, C.C., et al. 2004. Mechanisms of resistance to the cytotoxic effects of oxysterols in human leukemic cells. *J. Steroid Biochem. Mol. Biol.* 88: 311-320.
5. Ito, H., et al. 2006. Possible interaction of a Rho effector, Rhotekin, with a PDZ-protein, PIST, at synapses of hippocampal neurons. *Neurosci. Res.* 56: 165-171.

CHROMOSOMAL LOCATION

Genetic locus: RTKN2 (human) mapping to 10q21.2.

PRODUCT

Rhotekin 2 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 Rhotekin 2 shRNA Plasmid (h): sc-90523-SH and Rhotekin 2 shRNA (h) Lentiviral Particles: sc-90523-V as alternate gene silencing products.

For independent verification of Rhotekin 2 (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-90523A, sc-90523B and sc-90523C.

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

Rhotekin 2 siRNA (h) is recommended for the inhibition of Rhotekin 2 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

Rhotekin 2 (2C2): sc-517159 is recommended as a control antibody for monitoring of Rhotekin 2 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 Rhotekin 2 gene expression knockdown using RT-PCR Primer: Rhotekin 2 (h)-PR: sc-90523-PR (20 μ l). 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.