

RIC-8A siRNA (h): sc-76402

BACKGROUND

The Ras superfamily of GTPases can be subdivided into the Ras, Rho/Rac, Sar, Rab, Arf, Rap and Ran subfamilies, all of which control multiple aspects of cell function, including cytoskeletal rearrangement, nuclear signaling and cell growth. The Ras superfamily of GTPases function as regulated switches that toggle between a biologically active GTP-bound and an inactive GDP-bound form. This activation is catalyzed by guanine nucleotide exchange factors (GEFs). RIC-8A (resistance to inhibitors of cholinesterase 8 homolog A), also known as RIC8 or Synembryn-A, is a 530 amino acid cytoplasmic protein that can activate several G_{α} proteins, including $G_{\alpha i-1}$, $G_{\alpha q}$ and $G_{\alpha o}$. Functioning as a guanine nucleotide exchange factor, RIC-8A binds to GDP-associated substrates and exchanges bound GDP for free GTP. Via its ability to stimulate protein function, RIC-8A plays a role in regulating mitotic movement and may be involved in receptor-mediated ERK activation. RIC-8A is expressed as three isoforms due to alternative splicing events.

REFERENCES

1. Miller, K.G., et al. 2000. RIC-8 (Synembryn): a novel conserved protein that is required for $G_{\alpha q}$ signaling in the *C. elegans* nervous system. *Neuron* 27: 289-299.
2. Tall, G.G., et al. 2003. Mammalian RIC-8A (synembryn) is a heterotrimeric G_{α} protein guanine nucleotide exchange factor. *J. Biol. Chem.* 278: 8356-8362.
3. Klattenhoff, C., et al. 2003. Human brain synembryn interacts with $G_{\alpha s}$ and $G_{\alpha q}$ and is translocated to the plasma membrane in response to isoproterenol and carbachol. *J. Cell. Physiol.* 195: 151-157.
4. Malik, S., et al. 2005. RIC-8 enhances G protein $\beta\gamma$ -dependent signaling in response to $\beta\gamma$ -binding peptides in intact cells. *Mol. Pharmacol.* 68: 129-136.

CHROMOSOMAL LOCATION

Genetic locus: RIC8A (human) mapping to 11p15.5.

PRODUCT

RIC-8A 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 RIC-8A shRNA Plasmid (h): sc-76402-SH and RIC-8A shRNA (h) Lentiviral Particles: sc-76402-V as alternate gene silencing products.

For independent verification of RIC-8A (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-76402A, sc-76402B and sc-76402C.

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.

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

RIC-8A siRNA (h) is recommended for the inhibition of RIC-8A 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

RIC-8A (C-3): sc-398962 is recommended as a control antibody for monitoring of RIC-8A 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 RIC-8A gene expression knockdown using RT-PCR Primer: RIC-8A (h)-PR: sc-76402-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.