

RasGRP1 siRNA (h): sc-36397

BACKGROUND

The superfamily of GTP-binding proteins, of which Ras proteins are prototypes, has been implicated in a broad range of biological activities. Studies have identified a family of guanine nucleotide-releasing factors (GRFs) that activate Ras in mammalian cells and an "adapter" protein (Sem 5/GRB2) that appears to mediate the interaction of GRFs with activated receptor molecules. Subsequent to activation, Ras appears to interact with Raf, thereby activating the MAP kinase phosphorylation pathway. RasGRP1 is a guanyl nucleotide-releasing protein for Ras that contains two EF hand domains, which bind to calcium, and a diacylglycerol (DAG)-binding domain. RasGRP1 is expressed in the nervous system and lymphoid tissues and may link changes in DAG and calcium concentrations to Ras activation.

REFERENCES

1. Lowenstein, E.J., et al. 1992. The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. *Cell* 40: 431-442.
2. Chardin, P., et al. 1993. Human Sos 1: a guanine nucleotide exchange factor for ras that binds to GRB2. *Science* 260: 1338-1343.
3. Skolnik, E.Y., et al. 1993. The function of GRB2 in linking the Insulin receptor to ras signaling pathways. *Science* 260: 1953-1955.
4. Zhang, X., et al. 1993. Normal and oncogenic p21 Ras proteins bind to the amino-terminal regulatory domain of c-RAF-1. *Nature* 364: 308-313.
5. Ebinu, J.O., et al. 1998. RasGRP, a Ras guanyl nucleotide-releasing protein with calcium- and diacylglycerol-binding motifs. *Science* 280: 1082-1086.
6. Tognon, C.E., et al. 1998. Regulation of RasGRP via a phorbol ester-responsive C1 domain. *Mol. Cell. Biol.* 18: 6995-7008.

CHROMOSOMAL LOCATION

Genetic locus: RASGRP1 (human) mapping to 15q14.

PRODUCT

RasGRP1 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 RasGRP1 shRNA Plasmid (h): sc-36397-SH and RasGRP1 shRNA (h) Lentiviral Particles: sc-36397-V as alternate gene silencing products.

For independent verification of RasGRP1 (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-36397A, sc-36397B and sc-36397C.

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

RasGRP1 siRNA (h) is recommended for the inhibition of RasGRP1 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

RasGRP1 (199): sc-8430 is recommended as a control antibody for monitoring of RasGRP1 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 RasGRP1 gene expression knockdown using RT-PCR Primer: RasGRP1 (h)-PR: sc-36397-PR (20 μ l, 518 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.