

RasGRP4 siRNA (h): sc-97242

BACKGROUND

RasGRP4 (Ras guanyl releasing protein 4) is a member of the RasGRP family of guanine nucleotide-releasing factors (GRFs) that activate Ras in mammalian cells and are preferentially expressed in hematopoietic cells. RasGRP4 is a mast cell-specific protein that is widely expressed during fetal development and can be found in spleen, liver, skeletal muscle, placenta, heart and lung tissues of adults. Localizing to the cytoplasm, RasGRP4 contains one EF-hand domain which binds calcium, an N-terminal Ras-GEF domain and a phorbol-ester/DAG-type zinc finger which binds DAG (diacylglycerol). RasGRP4 functions as a DAG-regulated, cation-dependent, guanine nucleotide exchange factor (GEF). Upon activation by DAG, RasGRP4 is recruited to the plasma membrane where it is believed to participate in membrane ruffling and the spreading of activated cells. Mutations in the gene encoding RASGRP4 can result in a higher susceptibility to asthma and cancer.

REFERENCES

1. Yang, Y., et al. 2002. RasGRP4, a new mast cell-restricted Ras guanine nucleotide-releasing protein with calcium- and diacylglycerol-binding motifs. Identification of defective variants of this signaling protein in asthma, mastocytosis, and mast cell leukemia patients and demonstration of the importance of RasGRP4 in mast cell development and function. *J. Biol. Chem.* 277: 25756-25774.
2. Reuther, G.W., et al. 2002. RasGRP4 is a novel Ras activator isolated from acute myeloid leukemia. *J. Biol. Chem.* 277: 30508-30514.
3. Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 607320. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
4. Li, L., et al. 2003. RasGRP4 regulates the expression of prostaglandin D₂ in human and rat mast cell lines. *J. Biol. Chem.* 278: 4725-4729.
5. Li, L., et al. 2003. Mast cells in airway hypo-responsive C3H/HeJ mice express a unique isoform of the signaling protein Ras guanine nucleotide releasing protein 4 that is unresponsive to diacylglycerol and phorbol esters. *J. Immunol.* 171: 390-397.

CHROMOSOMAL LOCATION

Genetic locus: RASGRP4 (human) mapping to 19q13.2.

PRODUCT

RasGRP4 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 RasGRP4 shRNA Plasmid (h): sc-97242-SH and RasGRP4 shRNA (h) Lentiviral Particles: sc-97242-V as alternate gene silencing products.

For independent verification of RasGRP4 (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-97242A, sc-97242B and sc-97242C.

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

RasGRP4 siRNA (h) is recommended for the inhibition of RasGRP4 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

RasGRP4 (E-2): sc-515508 is recommended as a control antibody for monitoring of RasGRP4 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 RasGRP4 gene expression knockdown using RT-PCR Primer: RasGRP4 (h)-PR: sc-97242-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.

PROTOCOLS

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