SANTA CRUZ BIOTECHNOLOGY, INC.

RGS8 siRNA (h): sc-61472

BACKGROUND

The regulators of G protein signaling (RGS) proteins inhibit heterotrimeric G protein signaling. RGS proteins work by functioning as GTPase-activating proteins (which increase the GTPase activity of G protein α-subunits) thereby driving G proteins into their inactive GDP-bound form. RGS8 is a 180 amino acid RGS protein that is expressed mainly in the brain, specifically in the Purkinje cells of the cerebellum. RGS8 differs from most other RGS members in that RGS8 has a positive effect on G protein-coupled inwardly rectifying K^+ (GIRK1/2) channels, whereas other RGS proteins function as simple negative regulators. Because both positive and negative effects have been observed with the RGS8 protein, RGS8 expression most likely improves upon the kinetic efficacy of G-proteins. The NH_2 terminus of RGS8 is responsible for its subcellular localization.

REFERENCES

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2. Saitoh, O., et al. 1999. RGS7 and RGS8 differentially accelerate G protein-mediated modulation of K^+ currents. J. Biol. Chem. 274: 9899-9904.
3. Saitoh, O., et al. 2001. Regulator of G protein signaling 8 (RGS8) requires its NH_2 terminus for subcellular localization and acute desensitization of G protein-gated K^+ channels. J. Biol. Chem. 276: 5052-5058.
4. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 607189. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Saitoh, O., et al. 2002. Alternative splicing of RGS8 gene determines inhibitory function of receptor type-specific G_q signaling. Proc. Natl. Acad. Sci. USA 99: 10138-10143.
6. Saitoh, O. and Yoshihiro, K. 2004. Biochemical and electrophysiological analyses of RGS8 function. Methods Enzymol. 390: 129-148.
7. Benians, A., et al. 2004. Participation of RGS8 in the ternary complex of agonist, receptor and G protein. Biochem. Soc. Trans. 32: 1045-1047.
8. Kveberg, L., et al. 2005. Expression of regulator of G protein signalling cells, and their modulation by Ly49A and Ly49D. Immunology 115: 358-365.

CHROMOSOMAL LOCATION

Genetic locus: RGS8 (human) mapping to 1q25.3.

PRODUCT

RGS8 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see RGS8 shRNA Plasmid (h): sc-61472-SH and RGS8 shRNA (h) Lentiviral Particles: sc-61472-V as alternate gene silencing products.

For independent verification of RGS8 (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-61472A, sc-61472B and sc-61472C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

RGS8 siRNA (h) is recommended for the inhibition of RGS8 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 µM in 66 µl. 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

RGS8 (F-3): sc-398949 is recommended as a control antibody for monitoring of RGS8 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 RGS8 gene expression knockdown using RT-PCR Primer: RGS8 (h)-PR: sc-61472-PR (20 µl). 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.