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RGS5 siRNA (h): sc-45814

BACKGROUND

Regulators of G protein signaling (RGS proteins) are a family of highly diverse, multifunctional signaling proteins that share a conserved 120 amino acid domain (RGS domain). RGS domains bind directly to activated G_{α} subunits and act as GTPase-activating proteins (GAPs) to attenuate and/or modulate hormone and neurotransmitter receptor-initiated signaling by both G_{α} -GTP and $G_{\beta\gamma}$. RGS proteins shorten the lifetime of activated G proteins. Vascular endothelial cells express the RGS protein RGS5, where it correlates with capillary morphogenesis, thus rendering it a candidate gene involved in capillary growth, angiogenesis and also potentially the pathophysiology of stroke.

REFERENCES

1. Kardstuncer, T., et al. 1998. Cardiac myocytes express mRNA for ten RGS proteins: changes in RGS mRNA expression in ventricular myocytes and cultured atria. *FEBS Lett.* 438: 285-288.
2. Hepler, J.R. 1999. Emerging roles for RGS proteins in cell signalling. *Trends Pharmacol. Sci.* 20: 376-382.
3. Wieland, T., et al. 2003. Regulators of G protein signalling: multifunctional proteins with impact on signalling in the cardiovascular system. *Pharmacol. Ther.* 97: 95-115.
4. Bondjers, C., et al. 2003. Transcription profiling of platelet-derived growth factor-B-deficient mouse embryos identifies RGS5 as a novel marker for pericytes and vascular smooth muscle cells. *Am. J. Pathol.* 162: 721-729.
5. Cho, H., et al. 2003. Pericyte-specific expression of RGS5: implications for PDGF and EDG receptor signaling during vascular maturation. *FASEB J.* 17: 440-442.
6. Li, J., et al. 2004. Regulator of G protein signaling 5 marks peripheral arterial smooth muscle cells and is downregulated in atherosclerotic plaque. *J. Vasc. Surg.* 40: 519-528.
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CHROMOSOMAL LOCATION

Genetic locus: RGS5 (human) mapping to 1q23.3.

PRODUCT

RGS5 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 RGS5 shRNA Plasmid (h): sc-45814-SH and RGS5 shRNA (h) Lentiviral Particles: sc-45814-V as alternate gene silencing products.

For independent verification of RGS5 (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-45814A, sc-45814B and sc-45814C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

RGS5 siRNA (h) is recommended for the inhibition of RGS5 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

RGS5 (H-1): sc-390245 is recommended as a control antibody for monitoring of RGS5 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor RGS5 gene expression knockdown using RT-PCR Primer: RGS5 (h)-PR: sc-45814-PR (20 μ l, 541 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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