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RGS5 shRNA (m) Lentiviral Particles: sc-45815-V

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.

CHROMOSOMAL LOCATION

Genetic locus: *Rgs5* (mouse) mapping to 1 H3.

PRODUCT

RGS5 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see RGS5 siRNA (m): sc-45815 and RGS5 shRNA Plasmid (m): sc-45815-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

RGS5 shRNA (m) Lentiviral Particles is recommended for the inhibition of RGS5 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

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 (m)-PR: sc-45815-PR (20 μ l). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.