SANTA CRUZ BIOTECHNOLOGY, INC.

RGS9 siRNA (m): sc-36413

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The Power to Question

BACKGROUND

Heterotrimeric G proteins function to relay information from cell surface receptors to various intracellular effectors. G proteins comprise α, β and γ subunits, and following activation the a subunit binds GTP and dissociates from the $\beta\gamma$ complex. A large group of proteins have been identified as GTPase-activating proteins (GAPs), including the RGS (regulator of G protein signaling) family, which serve to deactivate specific G_α isoforms by increasing the rate at which they convert GTP to GDP. A subfamily of RGS proteins expressed in the central nervous system contain, in addition to the highly conserved RGS domain, a characteristic GGL domain, or G protein γ subunit-like domain, which mediates binding to $G_{\beta\ 5}$ subunits. This subfamily, which includes RGS6, RGS7, RGS9 and RGS11, associates with $G_{\beta\ 5}$ to form active GAP complexes that are predominantly localized to the cytosol. RGS/β 5 complexes preferentially target $G_{\alpha\ o}$ subunit for hydrolysis and inhibit $G_{\beta 1\gamma 2}$-mediated activation of phospholipase C.

REFERENCES

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2. Snow, B.E., et al. 1998. A G protein γ subunit-like domain shared between RGS11 and other RGS proteins specifies binding to $G_{\beta\ 5}$ subunits. Proc. Natl. Acad. Sci. USA 95: 13307-13312.
3. Thomas, E.A., et al. 1998. RGS9: a regulator of G-protein signalling with specific expression in rat and mouse striatum. J. Neurosci. Res. 52: 118-124.
4. Guan, K.L., et al. 1999. A G-protein signaling network mediated by an RGS protein. Genes and Dev. 13: 1763-1767.
5. Hepler, J.R. 1999. Emerging roles for RGS proteins in cell signaling. Trends Pharmacol. Sci. 20: 376-382.
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7. Snow, B.E., et al. 1999. Fidelity of G protein β-subunit association by the G protein gamma-subunit-like domains of RGS6, RGS7, and RGS11. Proc. Natl. Acad. Sci. USA 96: 6489-6494.

CHROMOSOMAL LOCATION

Genetic locus: Rgs9 (mouse) mapping to 11 E1.

PRODUCT

RGS9 siRNA (m) 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 RGS9 shRNA Plasmid (m): sc-36413-SH and RGS9 shRNA (m) Lentiviral Particles: sc-36413-V as alternate gene silencing products.

For independent verification of RGS9 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-36413A, sc-36413B and sc-36413C.

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

RGS9 siRNA (m) is recommended for the inhibition of RGS9 expression in mouse 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

RGS9 (C-8): sc-377252 is recommended as a control antibody for monitoring of RGS9 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 RGS9 gene expression knockdown using RT-PCR Primer: RGS9 (m)-PR: sc-36413-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.