

## RGS4 siRNA (m): sc-40664

### BACKGROUND

Heterotrimeric G proteins function to relay information from cell surface receptors to intracellular effectors. In mammals, G protein  $\alpha$ ,  $\beta$  and  $\gamma$  polypeptides are encoded by at least 16, 4 and 7 genes, respectively. Most interest in G proteins has been focused on their  $\alpha$  subunits, since these proteins bind and hydrolyze GTP and most obviously regulate the activity of the best studied effectors. Four  $G_{\alpha}$  GTPase-activating proteins (GAPs) have been identified and are designated RGS1 (regulator of G protein-signaling), RGS4, RGS10 and GAIP ( $G_{\alpha}$ -interacting protein). Each of these proteins has been shown to deactivate specific  $G_{\alpha}$  isoforms by increasing the rate at which they convert GTP to GDP. RGS1, RGS4 and GAIP bind tightly to and exhibit GAP activity towards  $G_{\alpha i}$ ,  $G_{\alpha o}$  and  $G_{\alpha t}$ , but not  $G_{\alpha s}$ . RGS10 increases the GTP hydrolytic activity of several members of the  $G_{\alpha i}$  subfamily, including  $G_{\alpha i-3}$ ,  $G_{\alpha z}$  and  $G_{\alpha o}$ .

### REFERENCES

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2. Cali, J.J., et al. 1992. Selective tissue distribution of G protein  $\gamma$  subunits, including a new form of the  $\gamma$  subunits identified by cDNA cloning. *J. Biol. Chem.* 267: 24023-24027.
3. McLaughlin, S.K., et al. 1992. Gustducin is a taste-cell-specific G protein closely related to the transducins. *Nature* 357: 563-569.
4. von Weizsacker, E., et al. 1992. Diversity among the  $\beta$  subunits of heterotrimeric GTP-binding proteins: characterization of a novel  $\beta$ -subunit cDNA. *Biochem. Biophys. Res. Commun.* 183: 350-356.
5. Kleuss, C., et al. 1992. Different  $\beta$ -subunits determine G protein interaction with transmembrane receptors. *Nature* 358: 424-426.
6. Conklin, B.R., et al. 1993. Structural elements of  $G_{\alpha}$  subunits that interact with  $G_{\beta\gamma}$  receptors, and effectors. *Cell* 73: 631-641.
7. Watson, N., et al. 1996. RGS family members: GTPase-activating proteins for heterotrimeric G-protein  $\alpha$ -subunits. *Nature* 383: 172-175.
8. Hunt, T.W., et al. 1996. RGS10 is a selective activator of  $G_{\alpha i}$  GTPase activity. *Nature* 383: 175-177.

### CHROMOSOMAL LOCATION

Genetic locus: *Rgs4* (mouse) mapping to 1 H3.

### PRODUCT

RGS4 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see RGS4 shRNA Plasmid (m): sc-40664-SH and RGS4 shRNA (m) Lentiviral Particles: sc-40664-V as alternate gene silencing products.

For independent verification of RGS4 (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-40664A, sc-40664B and sc-40664C.

### 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

### APPLICATIONS

RGS4 siRNA (m) is recommended for the inhibition of RGS4 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  $\mu$ M in 66  $\mu$ 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

RGS4 (H-12): sc-398348 is recommended as a control antibody for monitoring of RGS4 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

### RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor RGS4 gene expression knockdown using RT-PCR Primer: RGS4 (m)-PR: sc-40664-PR (20  $\mu$ l, 510 bp). 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](http://www.scbt.com) for detailed protocols and support products.