

# RGS6 siRNA (h): sc-40665

## BACKGROUND

Heterotrimeric G proteins function to relay information from cell surface receptors to various intracellular effectors. G proteins comprise  $\alpha$ ,  $\beta$  and  $\gamma$  subunits, and following activation the  $\alpha$  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  $G_{\alpha}$  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  $\gamma$  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/ $\beta 5$  complexes preferentially target  $G_{\alpha o}$  subunit for hydrolysis and inhibit  $G_{\beta 1\gamma 2}$ -mediated activation of phospholipase C.

## REFERENCES

- Conklin, B.R. and Bourne, H.R. 1993. Structural elements of  $G_{\alpha}$  subunits that interact with  $G_{\beta\gamma}$  receptors, and effectors. *Cell* 73: 631-641.
- Snow, B.E., et al. 1998. A G protein  $\gamma$  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.
- 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.
- Guan, K.L. and Han, M. 1999. A G protein signaling network mediated by an RGS protein. *Genes Dev.* 13: 1763-1767.
- Hepler, J.R. 1999. Emerging roles for RGS proteins in cell signaling. *Trends Pharmacol. Sci.* 20: 376-382.
- Posner, B.A., et al. 1999. Regulators of G protein signaling 6 and 7. Purification of complexes with  $G_{\beta 5}$  and assessment of their effects on G protein-mediated signaling pathways. *J. Biol. Chem.* 274: 31087-31093.
- Snow, B.E., et al. 1999. Fidelity of G protein  $\beta$ -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: RGS6 (human) mapping to 14q24.2.

## PRODUCT

RGS6 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see RGS6 shRNA Plasmid (h): sc-40665-SH and RGS6 shRNA (h) Lentiviral Particles: sc-40665-V as alternate gene silencing products.

For independent verification of RGS6 (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-40665A, sc-40665B and sc-40665C.

## 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

RGS6 siRNA (h) is recommended for the inhibition of RGS6 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  $\mu$ 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

RGS6/7 (F-10): sc-271643 is recommended as a control antibody for monitoring of RGS6 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 RGS6 gene expression knockdown using RT-PCR Primer: RGS6 (h)-PR: sc-40665-PR (20  $\mu$ l). 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.