SANTA CRUZ BIOTECHNOLOGY, INC.

RGS12 siRNA (h): sc-40671

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

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 the activated G protein. RGS12 is a GTPase-activating protein for G_t class α subunits. Rat cardiac myocytes express mRNA for at least 10 RGS proteins, including RGS12. RGS12 contains a Ras-binding domain (RBD), PDZ and PTB domains and single "LGN motifs" that are guanine nucleotide exchange factors specific for the α-subunit of G proteins. There are twelve distinct transcripts of human RGS12 that arise by unusually complex splicing of the RGS12 gene and are expressed at high levels in brain and lung and lower levels in testis, heart and spleen. The RGS gene generates proteins that are expressed in a tissue-specific manner and range in size from 356 to 1,447 amino acids. The human RGS12 gene maps to chromosome 4p16.3.

REFERENCES

1. Snow, B.E., et al. 1997. Molecular cloning and expression analysis of rat RGS12 and RGS14. Biochem. Biophys. Res. Commun. 233: 770-777.
2. Kardestuncer, 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.
3. Snow, B.E., et al. 1998. GTPase activating specificity of RGS12 and binding specificity of an alternatively spliced PDZ (PSD-95/Dlg/ZO-1) domain. J. Biol. Chem. 273: 17749-17755.

CHROMOSOMAL LOCATION

Genetic locus: RGS12 (human) mapping to 4p16.3.

PRODUCT

RGS12 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see RGS12 shRNA Plasmid (h): sc-40671-SH and RGS12 shRNA (h) Lentiviral Particles: sc-40671-V as alternate gene silencing products.

For independent verification of RGS12 (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-40671A, sc-40671B and sc-40671C.

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

RGS12 siRNA (h) is recommended for the inhibition of RGS12 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 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

RGS12 (G-4): sc-398545 is recommended as a control antibody for monitoring of RGS12 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 RGS12 gene expression knockdown using RT-PCR Primer: RGS12 (h)-PR: sc-40671-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Huang, J., et al. 2014. Inhibition of $G_{\alpha\,i}$ activity by $G_{\beta\gamma}$ is mediated by PI 3-kinase-γ- and cSrc-dependent tyrosine phosphorylation of $G_{\alpha\,i}$ and recruitment of RGS12. Am. J. Physiol. Gastrointest. Liver Physiol. 306: G802-G810.

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.