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GIT1 siRNA (r): sc-45954

BACKGROUND

Heterotrimeric G protein-mediated signal transduction is a dynamically regulated process with the intensity of signal decreasing over time despite the continued presence of the agonist. G protein-coupled receptor kinases (GRKs) are activated by activated G protein-coupled receptors, and they function to phosphorylate and inactivate cell surface receptors in the heterotrimeric G protein signaling cascade. GIT1 (for GRK-interactor 1) and GIT2 are GTPase-activating proteins (GAP) for members of the ADP ribosylation factor (ARF) family of small GTP-binding proteins, which are involved in vesicular trafficking. GIT1 overexpression results in reduced internalization and resensitization of β_2 -adrenergic receptor, thus reducing β_2 -adrenergic receptor signaling.

REFERENCES

1. Hausdorff, W.P., et al. 1990. Turning off the signal: desensitization of β -adrenergic receptor function. *FASEB J.* 4: 2881-2889.
2. Pei, G., et al. 1994. An approach to the study of G protein-coupled receptor kinases: an *in vitro*-purified membrane assay reveals differential receptor specificity and regulation by $G_{\beta\gamma}$ subunits. *Proc. Natl. Acad. Sci. USA* 91: 3633-3636.
3. Lefkowitz, R.J. 1998. G protein-coupled receptors. III. New roles for receptor kinases and β -arrestins in receptor signaling and desensitization. *J. Biol. Chem.* 273: 18677-18680.
4. Pitcher, J.A., et al. 1998. G protein-coupled receptor kinases. *Annu. Rev. Biochem.* 67: 653-692.

CHROMOSOMAL LOCATION

Genetic locus: *Git1* (rat) mapping to 10q26.

PRODUCT

GIT1 siRNA (r) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GIT1 shRNA Plasmid (r): sc-45954-SH and GIT1 shRNA (r) Lentiviral Particles: sc-45954-V as alternate gene silencing products.

For independent verification of GIT1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-45954A, sc-45954B and sc-45954C.

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

APPLICATIONS

GIT1 siRNA (r) is recommended for the inhibition of GIT1 expression in rat 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 μ M in 66 μ 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

GIT1 (A-1): sc-365084 is recommended as a control antibody for monitoring of GIT1 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 κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz MarkerTM Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 GIT1 gene expression knockdown using RT-PCR Primer: GIT1 (r)-PR: sc-45954-PR (20 μ l, 545 bp). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.

SELECT PRODUCT CITATIONS

1. Liu, S., et al. 2012. G protein-coupled receptor kinase interactor-1 (GIT1) is a new endothelial nitric-oxide synthase (eNOS) interactor with functional effects on vascular homeostasis. *J. Biol. Chem.* 287: 12309-12320.

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.