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GIT1 shRNA (r) Lentiviral Particles: sc-45954-V

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.
5. Premont, R.T., et al. 1998. β 2-Adrenergic receptor regulation by GIT1, a G protein-coupled receptor kinase-associated ADP ribosylation factor GTPase-activating protein. *Proc. Natl. Acad. Sci. USA* 95: 14082-14087.
6. Premont, R.T., et al. 2000. A second ARF GTPase-activating protein that interacts with GRKs. Functional diversity of GIT2 through alternative splicing. *J. Biol. Chem.* 275: 22373-22380.

CHROMOSOMAL LOCATION

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

PRODUCT

GIT1 shRNA (r) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see GIT1 siRNA (r): sc-45954 and GIT1 shRNA Plasmid (r): sc-45954-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

GIT1 shRNA (r) Lentiviral Particles is recommended for the inhibition of GIT1 expression in rat cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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