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GC-C shRNA (m) Lentiviral Particles: sc-45493-V

BACKGROUND

Guanylate cyclases belong to the adenylyl cyclase class-4/guanylyl cyclase family. There are two forms of guanylate cyclase, a soluble form (GCS or sGC), which act as receptors for nitric oxide and a membrane-bound receptor form (GC), which are peptide hormone receptors. The GC-C protein is composed of an extracellular domain, a single transmembrane domain and a cytoplasmic region consisting of a kinase-like domain and a catalytic domain. It is expressed as two differentially glycosylated forms, a precursor form, present in the endoplasmic reticulum, and a form present on the plasma membrane. Ligand binding to the extracellular domain of GC-C promotes the accumulation of cGMP. GC-C acts as the receptor for heat-stable enterotoxins, small peptides secreted by some pathogenic strains of *E. coli* that cause severe secretory diarrhea. GC-C also binds to guanylin and uroguanylin peptides, which modulate renal function in response to oral salt load.

REFERENCES

1. Denninger, J.W. and Marletta, M.A. 1999. Guanylate cyclase and the .NO/cGMP signaling pathway. *Biochim. Biophys. Acta* 1411: 334-350.
2. Condorelli, P., et al. 2001. *In vivo* control of soluble guanylate cyclase activation by nitric oxide: a kinetic analysis. *Biophys. J.* 80: 2110-2119.
3. Ghanekar, Y., et al. 2003. Cellular refractoriness to the heat-stable enterotoxin peptide is associated with alterations in levels of the differentially glycosylated forms of guanylyl cyclase C. *Eur. J. Biochem.* 270: 3848-3857.
4. Ghanekar, Y. et al. 2004. Glycosylation of the receptor guanylate cyclase C: role in ligand binding and catalytic activity. *Biochem. J.* 379: 653-663.
5. Nakauchi, M. and Suzuki, N. 2005. Enterotoxin/guanylin receptor type guanylyl cyclases in non-mammalian vertebrates. *Zool. Sci.* 22: 501-509.

CHROMOSOMAL LOCATION

Genetic locus: *Gucy2c* (mouse) mapping to 6 G1.

PRODUCT

GC-C shRNA (m) 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 GC-C siRNA (m): sc-45493 and GC-C shRNA Plasmid (m): sc-45493-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.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

APPLICATIONS

GC-C shRNA (m) Lentiviral Particles is recommended for the inhibition of GC-C expression in mouse 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

GCC1 (Q-15): sc-162850 is recommended as a control antibody for monitoring of GC-C 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor GC-C gene expression knockdown using RT-PCR Primer: GC-C (m)-PR: sc-45493-PR (20 μ l). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ 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.