

GC-C siRNA (h): sc-45492

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. *Zoolog. Sci.* 22: 501-509.
6. Kuhn, M., et al. 2005. Cardiac and intestinal natriuretic peptides: insights from genetically modified mice. *Peptides* 26: 1078-1085.

CHROMOSOMAL LOCATION

Genetic locus: GUCY2C (human) mapping to 12p13.1.

PRODUCT

GC-C 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GC-C shRNA Plasmid (h): sc-45492-SH and GC-C shRNA (h) Lentiviral Particles: sc-45492-V as alternate gene silencing products.

For independent verification of GC-C (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-45492A, sc-45492B and sc-45492C.

PROTOCOLS

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

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

GC-C siRNA (h) is recommended for the inhibition of GC-C 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 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

GC-C (537): sc-100302 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 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 Marker™ 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 GC-C gene expression knockdown using RT-PCR Primer: GC-C (h)-PR: sc-45492-PR (20 μ l, 540 bp). 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.