

# GCS- $\alpha$ -1 siRNA (h): sc-41010

## BACKGROUND

Guanylate cyclases belong to the adenylyl cyclase class-4/guanylyl cyclase family. There are two forms of guanylate cyclase. The soluble forms, known as GCS or sGC, act as receptors for nitric oxide. The membrane-bound receptor forms, known as GC, are peptide hormone receptors. GCS, a cGMP-synthesizing enzyme, is the major receptor for the neurotransmitter nitric oxide (NO). It plays a crucial role in smooth muscle contractility, platelet reactivity and neurotransmission. GCS is a heme containing heterodimer, consisting of one  $\alpha$  subunit, designated GCS- $\alpha$ -1, and one  $\beta$  subunit. The heme moiety mediates NO activation, and this heme group also binds carbon monoxide, which weakly stimulates the enzyme. Both NO and CO stimulation are enhanced by the allosteric activator 3-(5'-hydroxymethyl-2'-furyl)-benzyl-indazole, YC-1. YC-1 can also stimulate GCS in a NO-independent manner. Both the  $\alpha$  and  $\beta$  subunits are required for cGMP generation, and at least two isoforms exist for each subunit. Heterodimers consisting of  $\alpha$ -1/ $\beta$ -1 and  $\alpha$ -2/ $\beta$ -1 have been identified, and both display similar enzymatic activity.

## REFERENCES

1. Yuen, P., et al. 1990. A new form of guanylyl cyclase is preferentially expressed in rat kidney. *Biochemistry* 29: 10872-10878.
2. Wedel, B., et al. 1995. Functional domains of soluble guanylyl cyclase. *J. Biol. Chem.* 270: 24871-24875.
3. Bellamy, T., et al. 2000. Rapid desensitization of the nitric oxide receptor, soluble guanylyl cyclase, underlies diversity of cellular cGMP responses. *Proc. Natl. Acad. Sci. USA* 97: 2928-2933.
4. Lee, Y., et al. 2000. Human recombinant soluble guanylyl cyclase: expression, purification, and regulation. *Proc. Nat. Acad. Sci. USA* 97: 10763-10768.
5. Ibarra, C., et al. 2001. Regional and age-dependent expression of the nitric oxide receptor, soluble guanylyl cyclase, in the human brain. *Brain Res.* 907: 54-60.
6. Koblin, M., et al. 2001. Nitric oxide activates the  $\beta$ 2 subunit of soluble guanylyl cyclase in the absence of a second subunit. *J. Biol. Chem.* 276: 30737-30743.

## CHROMOSOMAL LOCATION

Genetic locus: GUCY1A3 (human) mapping to 4q32.1.

## PRODUCT

GCS- $\alpha$ -1 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GCS- $\alpha$ -1 shRNA Plasmid (h): sc-41010-SH and GCS- $\alpha$ -1 shRNA (h) Lentiviral Particles: sc-41010-V as alternate gene silencing products.

For independent verification of GCS- $\alpha$ -1 (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-41010A, sc-41010B and sc-41010C.

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

## APPLICATIONS

GCS- $\alpha$ -1 siRNA (h) is recommended for the inhibition of GCS- $\alpha$ -1 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  $\mu$ M in 66  $\mu$ 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

GCS- $\alpha$ -1 (D-9): sc-376502 is recommended as a control antibody for monitoring of GCS- $\alpha$ -1 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>TM</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor GCS- $\alpha$ -1 gene expression knockdown using RT-PCR Primer: GCS- $\alpha$ -1 (h)-PR: sc-41010-PR (20  $\mu$ l). 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.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.