SANTA CRUZ BIOTECHNOLOGY, INC.

GCS-β-1 siRNA (m): sc-36487

BACKGROUND

Guanylate cyclases belong to the adenylyl cyclase class-4/guanylyl cyclase family. There are two forms of guanylate cyclase. The soluble form, known as GCS or sGC, act as receptors for nitric oxide. The membrane-bound receptor form, known as GC, are peptide hormone receptors. GCS is a cGMP-synthesizing enzyme, which 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 α subunit and one β subunit. The heme moeity 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 α and β subunits are required for cGMP generation, and at least two isoforms exist for each subunit. Heterodimers consisting of α-1/β-1 and α-2/β-1 have been identified, and both display similar enzymatic activity. The distribution of the β-2 subunit seems to be much more restricted than the β-1 subunit, with predominant expression in kidney and liver.

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. Funcational 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 activates the β2 subunit of soluble guanylyl cyclase in the absence of a second subunit. J. Biol. Chem. 276: 30737-30743.

CHROMOSOMAL LOCATION

Genetic locus: Gucy1b3 (mouse) mapping to 3 E3.

PRODUCT

GCS-β-1 siRNA (m) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GCS-β-1 shRNA Plasmid (m): sc-36487-SH and GCS-β-1 shRNA (m) Lentiviral Particles: sc-36487-V as alternate gene silencing products.

For independent verification of GCS-β-1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-36487A, sc-36487B and sc-36487C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

GCS-β-1 siRNA (m) is recommended for the inhibition of GCS-β-1 expression in mouse 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 µM in 66 µl. 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-β-1 (G-3): sc-514183 is recommended as a control antibody for monitoring of GCS-β-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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 GCS-β-1 gene expression knockdown using RT-PCR Primer: GCS-β-1 (m)-PR: sc-36487-PR (20 µl, 438 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.

PROTOCOLS

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