

GCS- β -2 siRNA (h): sc-41014

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 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 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., Potter, L. and Garbers, D. 1990. A new form of guanylyl cyclase is preferentially expressed in rat kidney. *Biochemistry* 29: 10872-10878.
2. Wedel, B., Harteneck, C., Foerster, J., Friebe, A., Schultz, G. and Koesling, D. 1995. Functional domains of soluble guanylyl cyclase. *J. Biol. Chem.* 270: 24871-24875.
3. Bellamy, T., Wood, J., Goodwin, D. and Farthwaite, J. 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., Martin, E. and Murad, F. 2000. Human recombinant soluble guanylyl cyclase: expression, purification and regulation. *Proc. Natl. Acad. Sci. USA* 97: 10763-10768.
5. Ibarra, C., Nedvetsky, P., Gerlach, M., Riederer, P. and Schmidt, H. 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., Vehse, K., Budaeus, L., Scholz, H. and Behrends, S. 2001. Nitric oxide activates the β 2 subunit of soluble guanylyl cyclase in the absence of a second subunit. *J. Biol. Chem.* 276: 30737-30743.
7. Martin, E., Le, Y. and Murad, F. 2001. YC-1 activation of human soluble guanylyl cyclase has both heme-dependent and heme independent components. *Proc. Natl. Acad. Sci. USA* 98: 12938-12942.
8. Gibb, B. and Garthwaite, J. 2001. Subunits of nitric oxide receptor, soluble guanylyl cyclase, expressed in rat brain. *Eur. J. Neurosci.* 13: 539-544.

CHROMOSOMAL LOCATION

Genetic locus: GUCY1B2 (human) mapping to 13q14.2.

PROTOCOLS

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

PRODUCT

GCS- β -2 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 GCS- β -2 shRNA Plasmid (h): sc-41014-SH and GCS- β -2 shRNA (h) Lentiviral Particles: sc-41014-V as alternate gene silencing products.

For independent verification of GCS- β -2 (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-41014A, sc-41014B and sc-41014C.

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

GCS- β -2 siRNA (h) is recommended for the inhibition of GCS- β -2 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor GCS- β -2 gene expression knockdown using RT-PCR Primer: GCS- β -2 (h)-PR: sc-41014-PR (20 μ 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.