

## ROS-GC2 siRNA (h): sc-45441

### BACKGROUND

Guanylate cyclases belong to the adenylyl cyclase class-4/guanylyl cyclase family. Originally identified in bovine retina, rod outer segment membrane guanylate cyclase (ROS-GC) proteins are membrane bound cyclases that serve a key function in photoreceptor physiology. One unique feature of ROS-GCs is that they are not activated by extracellular peptide hormones, but are regulated by calmodulin-like  $\text{Ca}^{2+}$ -binding proteins GCAP1 and GCAP2. The GCAPs sense changes in intracellular  $\text{Ca}^{2+}$  concentration during illumination and activate ROS-GCs when the  $\text{Ca}^{2+}$  decreases below 500-600 nM in a dark adapted cell. One feature distinguishing the two forms of ROS-GCs is that ROS-GC1 has two  $\text{Ca}^{2+}$  switches and is regulated by GCAP1, whereas ROS-GC2 has only one switch and is regulated by GCAP2. ROS-GC2 also contains a unique stretch of 5 amino acids on its C-terminus which is not present in ROS-GC1.

### REFERENCES

1. Goraczniak, R., et al. 1997. Structural and functional characterization of a second subfamily member of the calcium-modulated bovine rod outer segment membrane guanylate cyclase, ROS-GC2. *Biochem. Biophys. Res. Commun.* 234: 666-670.
2. Duda, T., et al. 1998. Differential activation of rod outer segment membrane guanylate cyclases, ROS-GC1 and ROS-GC2, by CD-GCAP and identification of the signaling domain. *Biochem. Biophys. Res. Commun.* 242: 118-122.
3. Duda, T., et al. 1998. Rod outer segment membrane guanylate cyclase type 1 (ROS-GC1) gene: structure, organization and regulation by phorbol ester, a protein kinase C activator. *Mol. Cell. Biochem.* 189: 63-70.
4. Goraczniak, R.M., et al. 1998. Calcium modulated signaling site in type 2 rod outer segment membrane guanylate cyclase (ROS-GC2). *Biochem. Biophys. Res. Commun.* 245: 447-453.
5. Kumar, V.D., et al. 1999. A second calcium regulator of rod outer segment membrane guanylate cyclase, ROS-GC1: neurocalcin. *Biochemistry* 38: 12614-12620.
6. Venkataraman, V., et al. 2000. Rod outer segment membrane guanylate cyclase type 1-linked stimulatory and inhibitory calcium signaling systems in the pineal gland: biochemical, molecular, and immunohistochemical evidence. *Biochemistry* 39: 6042-6052.
7. Koch, K.W., et al. 2002. Photoreceptor specific guanylate cyclases in vertebrate phototransduction. *Mol. Cell. Biochem.* 230: 97-106.
8. Subbaraya, I., et al. 2003. Structure and  $\text{Ca}^{2+}$  regulation of frog photoreceptor guanylate cyclase, ROS-GC1. *Mol. Cell. Biochem.* 254: 9-19.

### CHROMOSOMAL LOCATION

Genetic locus: GUCY2F (human) mapping to Xq22.3.

### 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.

### PRODUCT

ROS-GC2 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  $\mu\text{M}$  solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ROS-GC2 shRNA Plasmid (h): sc-45441-SH and ROS-GC2 shRNA (h) Lentiviral Particles: sc-45441-V as alternate gene silencing products.

For independent verification of ROS-GC2 (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-45441A, sc-45441B and sc-45441C.

### STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at  $-20^{\circ}\text{C}$  with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at  $-20^{\circ}\text{C}$ , avoid contact with RNAses and repeated freeze thaw cycles.

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

### APPLICATIONS

ROS-GC2 siRNA (h) is recommended for the inhibition of ROS-GC2 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  $\mu\text{M}$  in 66  $\mu\text{l}$ . 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 ROS-GC2 gene expression knockdown using RT-PCR Primer: ROS-GC2 (h)-PR: sc-45441-PR (20  $\mu\text{l}$ ). Annealing temperature for the primers should be  $55-60^{\circ}\text{C}$  and the extension temperature should be  $68-72^{\circ}\text{C}$ .