SANTA CRUZ BIOTECHNOLOGY, INC.

ROS-GC1 siRNA (m): sc-45430

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) and a membrane-bound receptor form. Rod outer segment membrane guanylate cyclase (ROS-GC) is a critical component of the vertebrate phototransduction machinery. ROS-GC1 is present in the retinal tissue and is localized exclusively in the nuclei and inner segments of the rod and cone photoreceptor cells. Defects in GUCY2D, the gene encoding ROS-GC1, are a cause of dominant cone-rod dystrophy type 6 (CORD6). CORD6 disease is characterized by the initial degeneration of cone photoreceptor cells, causing early loss of visual acuity and color vision, followed by the degeneration of rod photoreceptor cells leading to progressive night blindness and peripheral visual field loss.

REFERENCES

1. Kumar, V.D., et al. 1999. A second calcium regulator of rod outer segment membrane guanylate cyclase, ROS-GC1: neurocalcin. Biochemistry 38: 12614-12620.
2. Denninger, J.W., et al. 1999. Guanylate cyclase and the NO/cGMP signaling pathway. Biochim. Biophys. Acta 1411: 334-350.
3. 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.
4. Condorelli, P., et al. 2001. *In vivo* control of soluble guanylate cyclase activation by nitric oxide: a kinetic analysis. Biophys. J. 80: 2110-2119.
5. Subbaraya, I., et al. 2003. Structure and Ca^{2+} regulation of frog photoreceptor guanylate cyclase, ROS-GC1. Mol. Cell. Biochem. 254: 9-19.
6. SWISS-PROT/TrEMBL (Q02846). World Wide Web URL: http://www.expasy.ch/sprot/sprot-top.html.

CHROMOSOMAL LOCATION

Genetic locus: Gucy2e (mouse) mapping to 11 B3.

PRODUCT

ROS-GC1 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 ROS-GC1 shRNA Plasmid (m): sc-45430-SH and ROS-GC1 shRNA (m) Lentiviral Particles: sc-45430-V as alternate gene silencing products.

For independent verification of ROS-GC1 (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-45430A, sc-45430B and sc-45430C.

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 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

ROS-GC1 siRNA (m) is recommended for the inhibition of ROS-GC1 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

ROS-GC1 (B-7): sc-376217 is recommended as a control antibody for monitoring of ROS-GC1 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 ROS-GC1 gene expression knockdown using RT-PCR Primer: ROS-GC1 (m)-PR: sc-45430-PR (20 µl). 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.