

GCAP1 siRNA (m): sc-40629

BACKGROUND

The intracellular stimulation of guanylate cyclase (GC) by calcium, a key event in the recovery of the dark state of rod photoreceptors after exposure to light, is mediated by guanylate cyclase-activating protein (GCAP1). GCAPs are calcium-binding proteins belonging to the calmodulin superfamily. GCAP1 is a calcium-binding protein that stimulates synthesis of c-GMP in photoreceptors. GCAP1 is present in rod and cone photoreceptor outer segments where phototransduction occurs. In contrast to other calcium-binding proteins from the calmodulin superfamily, the calcium-free form of GCAP1 stimulates the effector enzyme. By molecular cloning of human and mouse GCAP cDNA, the known mammalian GCAPs are found to be more than 90% similar, consisting of 201 to 205 amino acids, and containing three identically conserved calcium-binding sites. A related protein, GCAP2, is detectable only in the retina and results from a gene duplication event.

REFERENCES

1. Subbaraya, I., et al. 1994. Molecular characterization of human and mouse photoreceptor guanylate cyclase-activating protein (GCAP) and chromosomal localization of the human gene. *J. Biol. Chem.* 269: 31080-31089.
2. Gorczyca, W.A., et al. 1995. Guanylyl cyclase activating protein. A calcium-sensitive regulator of phototransduction. *J. Biol. Chem.* 270: 22029-22036.
3. Otto-Bruc, A., et al. 1997. Localization of guanylate cyclase-activating protein 2 in mammalian retinas. *Proc. Natl. Acad. Sci. USA* 94: 4727-4732.
4. Surguchov, A., et al. 1997. The human GCAP1 and GCAP2 genes are arranged in a tail-to-tail array on the short arm of chromosome 6p21.1. *Genomics* 39: 312-322.
5. Rudnicka-Nawrot, M., et al. 1998. Changes in biological activity and folding of guanylate cyclase-activating protein 1 as a function of calcium. *Biochemistry* 37: 248-257.
6. Sokal, I., et al. 1999. Conformational changes in guanylyl cyclase-activating protein 1 (GCAP1) and its tryptophan mutants as a function of calcium concentration. *J. Biol. Chem.* 274: 19829-19837.
7. LocusLink Report (LocusID: 600364). <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: Guca1a (mouse) mapping to 17 C.

PRODUCT

GCAP1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GCAP1 shRNA Plasmid (m): sc-40629-SH and GCAP1 shRNA (m) Lentiviral Particles: sc-40629-V as alternate gene silencing products.

For independent verification of GCAP1 (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-40629A, sc-40629B and sc-40629C.

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

GCAP1 siRNA (m) is recommended for the inhibition of GCAP1 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 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.

GENE EXPRESSION MONITORING

GCAP1 (E-5): sc-390695 is recommended as a control antibody for monitoring of GCAP1 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 κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 GCAP1 gene expression knockdown using RT-PCR Primer: GCAP1 (m)-PR: sc-40629-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.

PROTOCOLS

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