SANTA CRUZ BIOTECHNOLOGY, INC.

rhodopsin siRNA (m): sc-40151

BACKGROUND

G protein-coupled receptors (GPCRs), which are characterized as containing seven transmembrane α helices, elicit G protein-mediated signaling cascades in response to a variety of stimuli. The opsin subfamily, which represents approximately 90 percent of all GPCRs, is comprised of photoreceptors that are activated by light, which include the red-, green- and blue-sensitive opsins and rhodopsin. The opsin subfamily consists of an apoprotein covalently linked to 11-*cis*-retinal, which undergoes isomerization upon the absorption of photons. This isomerization leads to a conformational change of the protein which results in the activation of hundreds of G proteins. Specifically, rhodopsin exhibits a maximal absorption at 495 nm and mediates vision in dim light. Mutations in the rhodopsin gene lead to retinitis pigmentosa, which can be inherited as an autosomal dominant, an autosomal recessive or an X-linked recessive disorder.

REFERENCES

1. Fung, B.K., et al. 1980. Flow of information in the light-triggered cyclic nucleotide cascade of vision. Proc. Natl. Acad. Sci. USA 78: 152-156.
2. Hargrave, P.A., et al. 1983. The structure of bovine rhodopsin. Biophys. Struct. Mech. 9: 235-244.
3. Wang, S.Z., et al. 1992. A visual pigment from chicken that resembles rhodopsin: amino acid sequence, gene structure and functional expression. Biochemistry 13: 3309-3315.
4. al-Maghtheh, M., et al. 1993. Rhodopsin mutations in autosomal dominant retinitis pigmentosa. Hum. Mutat. 2: 249-255.
5. Iiri, T., et al. 1998. G-protein diseases furnish a model for the turn-on switch. Nature 394: 35-38.
6. Lindsay, S.M., et al. 1999. Spectral sensitivity of vision and bioluminescence in the midwater shrimp. Biol. Bull.197: 348-360.
7. Palczewski, K., et al. 2000. Crystal structure of rhodopsin: a G protein-coupled receptor. Science 289: 739-745.

CHROMOSOMAL LOCATION

Genetic locus: (mouse) mapping to 6 E3.

PRODUCT

rhodopsin 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 rhodopsin shRNA Plasmid (m): sc-40151-SH and rhodopsin shRNA (m) Lentiviral Particles: sc-40151-V as alternate gene silencing products.

For independent verification of rhodopsin (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-40151A, sc-40151B and sc-40151C.

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

rhodopsin siRNA (m) is recommended for the inhibition of rhodopsin 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

rhodopsin (1D4): sc-57432 is recommended as a control antibody for monitoring of rhodopsin 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 rhodopsin gene expression knockdown using RT-PCR Primer: rhodopsin (m)-PR: sc-40151-PR (20 µl, 594 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.