SANTA CRUZ BIOTECHNOLOGY, INC.

melanopsin siRNA (m): sc-40147

BACKGROUND

G protein coupled receptors (GPCRs) contain seven transmembrane helices and elicit G protein-mediated signaling cascades. The opsin family represents approximately 90 percent of all GPCRs and includes red, green, and blue-sensitive opsins, rhodopsin and melanopsin. Opsins consist of an apoprotein covalently linked to 11-*cis*-retinal that undergoes isomerization upon photon absorption. The photon-induced conformation change of opsin activates hundreds of G proteins. Mammalian melanopsin expression selectively occurs in the inner retina and not in the photoreceptor cells critical for vision. Melanopsin plays a nonessential role in the transduction of photic stimuli for light/dark entrainment.

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. Iiri, T., et al. 1998. G protein diseases furnish a model for the turn-on switch. Nature 394: 35-38.
4. Palczewski, K., et al. 2000. Crystal Structure of Rhodopsin: A G protein-coupled receptor. Science 289: 739-745.
5. Provencio, I., et al. 2000. A novel human opsin in the inner retina. J. Neurosci. 20:600-605.
6. Ruby, N.F., et al. 2002. Role of melanopsin in circadian responses to light. Science 298: 2211-2213.

CHROMOSOMAL LOCATION

Genetic locus: Opn4 (mouse) mapping to 14 B.

PRODUCT

melanopsin 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 melanopsin shRNA Plasmid (m): sc-40147-SH and melanopsin shRNA (m) Lentiviral Particles: sc-40147-V as alternate gene silencing products.

For independent verification of melanopsin (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-40147A, sc-40147B and sc-40147C.

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

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

melanopsin (E-12): sc-515838 is recommended as a control antibody for monitoring of melanopsin 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 melanopsin gene expression knockdown using RT-PCR Primer: melanopsin (m)-PR: sc-40147-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.

PROTOCOLS

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