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RPE65 siRNA (h): sc-44898

BACKGROUND

The retinal pigment epithelium (RPE) is a monolayer simple epithelium in proximity to the outer surface of the retinal photoreceptor cells. Retinal pigment epithelium-specific protein (RPE65) is a 65 kDa protein belonging to the β -carotene dioxygenase family. This protein is important in 11-*cis* retinal production as well as in visual pigment regeneration. RPE65 is attached to the membrane by a lipid anchor when palmitoylated (membrane form) and soluble when unpalmitoylated. The soluble form of the protein binds vitamin A. Defects in RPE65 causes autosomal dominant retinitis pigmentosa and/or Leber congenital amaurosis type 2.

REFERENCES

- Hamel, C.P., et al. 1993. Molecular cloning and expression of RPE65, a novel retinal pigment epithelium-specific microsomal protein that is post-transcriptionally regulated *in vitro*. *J. Biol. Chem.* 268: 15751-15757.
- Hamel, C.P., et al. 1994. The gene for the retinal pigment epithelium-specific protein RPE65 is localized to human 1p31 and mouse 3. *Genomics* 20: 509-512.
- Morimura, H., et al. 1998. Mutations in the RPE65 gene in patients with autosomal recessive retinitis pigmentosa or Leber congenital amaurosis. *Proc. Natl. Acad. Sci. USA* 95: 3088-3093.
- Thompson, D.A., et al. 2000. Genetics and phenotypes of RPE65 mutations in inherited retinal degeneration. *Invest. Ophthalmol. Vis. Sci.* 41: 4293-4299.
- Seeliger, M.W., et al. 2001. New views on RPE65 deficiency: the rod system is the source of vision in a mouse model of Leber congenital amaurosis. *Nat. Genet.* 29: 70-74.

CHROMOSOMAL LOCATION

Genetic locus: RPE65 (human) mapping to 1p31.3.

PRODUCT

RPE65 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see RPE65 shRNA Plasmid (h): sc-44898-SH and RPE65 shRNA (h) Lentiviral Particles: sc-44898-V as alternate gene silencing products.

For independent verification of RPE65 (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-44898A, sc-44898B and sc-44898C.

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.

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

RPE65 siRNA (h) is recommended for the inhibition of RPE65 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 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

RPE65 (E-5): sc-390787 is recommended as a control antibody for monitoring of RPE65 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 RPE65 gene expression knockdown using RT-PCR Primer: RPE65 (h)-PR: sc-44898-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.