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### SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

[mail@szabo-scandic.com](mailto:mail@szabo-scandic.com)

[www.szabo-scandic.com](http://www.szabo-scandic.com)

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 



# RPE65 shRNA (h) Lentiviral Particles: sc-44898-V

## 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  $\beta$ -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.
- Rohrer, B., et al. 2003. Correlation of regenerable opsin with rod ERG signal in Rpe65<sup>-/-</sup> mice during development and aging. *Invest. Ophthalmol. Vis. Sci.* 44: 310-315.

## CHROMOSOMAL LOCATION

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

## PRODUCT

RPE65 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see RPE65 siRNA (h): sc-44898 and RPE65 shRNA Plasmid (h): sc-44898-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^{\circ}$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^{\circ}$  C for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

RPE65 shRNA (h) Lentiviral Particles is recommended for the inhibition of RPE65 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## 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  $\mu$ l). Annealing temperature for the primers should be  $55-60^{\circ}$  C and the extension temperature should be  $68-72^{\circ}$  C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

## PROTOCOLS

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