SANTA CRUZ BIOTECHNOLOGY, INC.

PPEF-1 siRNA (h): sc-106436

BACKGROUND

X-linked juvenile retinoschisis is a progressive vitreoretinal degeneration that maps to human chromosome Xp22.13. The gene, PPEF, is highly homologous to the retinal degeneration gene C (rdgC) found in *Drosophila melanogaster,* which is required to prevent light-induced retinal degeneration. PPEF-1 (protein protease with EF-hand motifs 1), a serine/threonine protein phosphatase, is expressed in the brain, with particularly high expression seen in fetal brain. Specifically, PPEF is highly expressed in sensory neurons of the dorsal root ganglia (DRG) and neural crest-derived cranial ganglia. Another homolog, PPEF-2, maps to human chromosome 4q21.1. In the adult, PPEF-2 is expressed specifically in retinal rod photoreceptors and the pineal. In the retina, several isoforms of PPEF-2 are predicted to arise from differential splicing. Through gene disruption studies, it has been determined that in contrast to loss of rdgC function in *Drosophila,* elimination of PPEF function does not cause retinal degeneration in vertebrates.

REFERENCES

1. Montini, E., Rugarli, E.I., Van de Vosse, E., Andolfi, G., Mariani, M., Puca, A.A., Consalez, G.G., den Dunnen, J.T., Ballabio, A. and Franco, B. 1997. A novel human serine-threonine phosphatase related to the *Drosophila* retinal degeneration C (rdgC) gene is selectively expressed in sensory neurons of neural crest origin. Hum. Mol. Genet. 6: 1137-1145.
2. Sherman, P.M., Sun, H., Macke, J.P., Williams, J., Smallwood, P.M. and Nathans, J. 1997. Identification and characterization of a conserved family of protein serine/threonine phosphatases homologous to *Drosophila* retinal degeneration C. Proc. Natl. Acad. Sci. USA 94: 11639-11644.
3. Ramulu, P., Kennedy, M., Xiong, W.H., Williams, J., Cowan, M., Blesh, D., Yau, K.W., Hurley, J.B. and Nathans, J. 2001. Normal light response, photoreceptor integrity, and rhodopsin dephosphorylation in mice lacking both protein phosphatases with EF hands (PPEF-1 and PPEF-2). Mol. Cell. Biol. 21: 8605-8614.
4. LocusLink Report (LocusID: 300109). http://www.ncbi.nlm.nih.gov/LocusLink/

CHROMOSOMAL LOCATION

Genetic locus: PPEF1 (human) mapping to Xp22.13.

PRODUCT

PPEF-1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PPEF-1 shRNA Plasmid (h): sc-106436-SH and PPEF-1 shRNA (h) Lentiviral Particles: sc-106436-V as alternate gene silencing products.

For independent verification of PPEF-1 (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-106436A, sc-106436B and sc-106436C.

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

PPEF-1 siRNA (h) is recommended for the inhibition of PPEF-1 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 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

PPEF-1 (SW-67): sc-134424 is recommended as a control antibody for monitoring of PPEF-1 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 PPEF-1 gene expression knockdown using RT-PCR Primer: PPEF-1 (h)-PR: sc-106436-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.