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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

www.szabo-scandic.com

linkedin.com/company/szaboscandic

SANTA CRUZ BIOTECHNOLOGY, INC.

PDE6β siRNA (m): sc-152131

BACKGROUND

Cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase (PDE6) plays a crucial role in the phototransduction cascade in the vertebrate retina. The enzyme consists of an α and a β subunit, with catalytic and cGMP binding activity, respectively, as well as two inhibitory γ subunits and a δ subunit. PDE6 reduces intracellular cytoplasmic cGMP levels, specifically in photoreceptor cells. Mutations in the human PDE6A gene, which encodes the α subunit, account for roughly 3-4% of the cases of recessive retinitis pigmentosa (RP) in North America.

REFERENCES

1. Mohamed, M.K., Taylor, R.E., Feinstein, D.S., Huang, X. and Pittler, S.J. 1998. Structure and upstream region characterization of the human gene encoding rod photoreceptor cGMP phosphodiesterase α-subunit. J. Mol. Neurosci. 10: 235-250.
2. Dryja, T.P., Rucinski, D.E., Chen, S.H. and Berson, E.L. 1999. Frequency of mutations in the gene encoding the α subunit of rod cGMP-phosphodiesterase in autosomal recessive retinitis pigmentosa. Invest. Ophthalmol. Vis. Sci. 40: 1859-1865.
3. Dekomien, G. and Epplen, J.T. 2000. Exclusion of the PDE6A gene for generalised progressive retinal atrophy in 11 breeds of dog. Anim. Genet. 31: 135-139.
4. Pittler, S.J., Zhang, Y., Chen, S., Mears, A.J., Zack, D.J., Ren, Z., Swain, P.K., Yao, S., Swaroop, A. and White, J.B. 2004. Functional analysis of the rod photoreceptor cGMP phosphodiesterase α-subunit gene promoter: Nrl and Crx are required for full transcriptional activity. J. Biol. Chem. 279: 19800-19807.

CHROMOSOMAL LOCATION

Genetic locus: Pde6b (mouse) mapping to 5 F.

PRODUCT

PDE6β 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 PDE6β shRNA Plasmid (m): sc-152131-SH and PDE6β shRNA (m) Lentiviral Particles: sc-152131-V as alternate gene silencing products.

For independent verification of PDE6β (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-152131A, sc-152131B and sc-152131C.

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

PDE6β siRNA (m) is recommended for the inhibition of PDE6β 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

PDE6β (B-8): sc-377486 is recommended as a control antibody for monitoring of PDE6β 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 PDE6β gene expression knockdown using RT-PCR Primer: PDE6β (m)-PR: sc-152131-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 or our catalog for detailed protocols and support products.