SANTA CRUZ BIOTECHNOLOGY, INC.

Optineurin siRNA (r): sc-60013

BACKGROUND

Optineurin, also designated FIP2, E3-14.7K-interacting protein, HYPL, transcription factor IIIA-interacting protein (TFIIIA-INTP), Huntingtin interacting protein L and NEMO-related protein, influences cell morphogenesis, membrane trafficking, vesicle trafficking and transcription activation through its interactions with the Rab8, Huntingtin and transcription factor IIIA proteins. Optineurin interacts with adenovirus E3-14.7K protein and may utilize TNFα or FAS-ligand pathways to mediate apoptosis, inflammation or vasoconstriction. Optineurin mutations may impart normal-tension glaucoma and adult-onset primary open angle glaucoma. Optineurin is a 617 amino acid protein that contains leucine zippers and leucine-rich regions, and contains a potential Cys2-His-Cys zinc finger at residues 553-582. It localizes to the Golgi apparatus. RT-PCR studies indicate expression in human trabecular meshwork, nonpigmented ciliary epithelium, retina, brain, adrenal cortex, liver, fetus, lymphocyte and fibroblast. Northern blot studies indicate a 2.0 kb transcript in human trabecular meshwork and nonpigmented ciliary epithelium and a minor 3.6 kb transcript.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 602432. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
2. Rezaie, T., et al. 2005. Molecular cloning and expression profiling of optineurin in the rhesus monkey. Invest. Ophthalmol. Vis. Sci. 46: 2404-2410.
3. Aung, T., et al. 2005. Clinical features and course of patients with glaucoma with the E50K mutation in the optineurin gene. Invest. Ophthalmol. Vis. Sci. 46: 2816-2822.
4. Jansson, M., et al. 2005. Analysis of rare variants and common haplotypes in the Optineurin gene in Swedish glaucoma cases. Ophthalmic Genet. 26: 85-89.
5. Rezaie, T., et al. 2005. Molecular cloning, genomic structure and protein characterization of mouse Optineurin. Genomics 85: 131-138.
6. Sahlender, D.A., et al. 2005. Optineurin links Myosin VI to the Golgi complex and is involved in Golgi organization and exocytosis. J. Cell Biol. 169: 285-295.

CHROMOSOMAL LOCATION

Genetic locus: Optn (rat) mapping to 17q12.3.

PRODUCT

Optineurin siRNA (r) 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 Optineurin shRNA Plasmid (r): sc-60013-SH and Optineurin shRNA (r) Lentiviral Particles: sc-60013-V as alternate gene silencing products.

For independent verification of Optineurin (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-60013A, sc-60013B and sc-60013C.

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

Optineurin siRNA (r) is recommended for the inhibition of Optineurin expression in rat 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

Optineurin (C-2): sc-166576 is recommended as a control antibody for monitoring of Optineurin 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 Optineurin gene expression knockdown using RT-PCR Primer: Optineurin (r)-PR: sc-60013-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.