

Peroxin 5R siRNA (h): sc-77918

BACKGROUND

Peroxisomes are single-membrane bound organelles present in virtually all eukaryotic cells. They are involved in numerous catabolic and anabolic pathways, including β -oxidation of very long chain fatty acids, metabolism of hydrogen peroxide, plasmalogen biosynthesis and bile acid synthesis. The Peroxin gene family, which includes more than 20 members, is required for peroxisome biogenesis. Peroxin 5R, also known as PEX5-related protein or Peroxisome biogenesis factor 5-like, is a 626 amino acid protein that is mainly expressed in brain, with some expression in testis and pancreas. Peroxin 5R contains five TPR repeats, which enable protein-protein interactions and assembly of large multiprotein complexes. There are three isoforms of Peroxin 5R that are produced as a result of alternative splicing events. These isoforms bind C-terminal peroxisome-targeting signals in a similar manner to Peroxin-5. Peroxin 5R interacts with Rab 8b, possibly playing a role in vesicular trafficking and neurotransmitter release.

REFERENCES

1. Fujiki, Y. 2000. Peroxisome biogenesis and peroxisome biogenesis disorders. *FEBS Lett.* 476: 42-46.
2. Otera, H., et al. 2000. The mammalian peroxin Pex5pL, the longer isoform of the mobile peroxisome targeting signal (PTS) type 1 transporter, translocates the Pex7p.PTS2 protein complex into peroxisomes via its initial docking site, Pex14p. *J. Biol. Chem.* 275: 21703-21714.
3. Matsumura, T., et al. 2000. Disruption of the interaction of the longer isoform of Pex5p, Pex5pL, with Pex7p abolishes peroxisome targeting signal type 2 protein import in mammals. Study with a novel Pex5-impaired Chinese hamster ovary cell mutant. *J. Biol. Chem.* 275: 21715-21721.
4. Amery, L., et al. 2001. Identification of PEX5p-related novel peroxisome-targeting signal 1 (PTS1)-binding proteins in mammals. *Biochem. J.* 357: 635-646.
5. Otera, H., et al. 2002. Peroxisomal targeting signal receptor Pex5p interacts with cargoes and import machinery components in a spatiotemporally differentiated manner: conserved Pex5p WXXXF/Y motifs are critical for matrix protein import. *Mol. Cell. Biol.* 22: 1639-1655.

CHROMOSOMAL LOCATION

Genetic locus: PEX5L (human) mapping to 3q26.33.

PRODUCT

Peroxin 5R 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 Peroxin 5R shRNA Plasmid (h): sc-77918-SH and Peroxin 5R shRNA (h) Lentiviral Particles: sc-77918-V as alternate gene silencing products.

For independent verification of Peroxin 5R (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-77918A, sc-77918B and sc-77918C.

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

Peroxin 5R siRNA (h) is recommended for the inhibition of Peroxin 5R 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

Peroxin 5R (A-4): sc-398580 is recommended as a control antibody for monitoring of Peroxin 5R 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 MarkerTM 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 Peroxin 5R gene expression knockdown using RT-PCR Primer: Peroxin 5R (h)-PR: sc-77918-PR (20 μ l). 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.