

OCRL siRNA (m): sc-39074

BACKGROUND

The inositol polyphosphate 5-phosphatases selectively remove the phosphate from the 5-position of various phosphatidylinositols, which generate second messengers in response to extracellular signals. OCRL1 is a type II 5-phosphatase that is mutated in the oculocerebrorenal syndrome of Lowe (OCRL). OCRL is a rare X-linked disorder that is characterized in part by congenital cataracts, mental retardation, muscular hypotonia, and renal tubular dysfunction. OCRL1 has a high affinity for phosphatidylinositol 4,5-bisphosphate as well as inositol 1,4,5-trisphosphate, and inositol 1,3,4,5-tetrakisphosphate as substrates. OCRL1 is localized to the Golgi complex and is thought to be part of the trans-Golgi network (TGN), which suggests that OCRL1 plays a role in protein sorting and trafficking within the cell.

REFERENCES

1. Zhang, X., et al. 1995. The protein deficient in Lowe syndrome is a phosphatidylinositol-4,5-bisphosphate 5-phosphatase. *Proc. Natl. Acad. Sci. USA* 92: 4853-4856.
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3. Zhang, X., et al. 1998. Phosphatidylinositol signalling reactions. *Semin. Cell Dev. Biol.* 9: 153-160.
4. Erneux, C., et al. 1998. The diversity and possible functions of the inositol polyphosphate 5-phosphatases. *Biochim. Biophys. Acta* 1436: 185-199.
5. Majerus, P.W., et al. 1999. The role of phosphatases in inositol signaling reactions. *J. Biol. Chem.* 274: 10669-10672.
6. Roschinger, W., et al. 2000. Carrier assessment in families with Lowe oculocerebrorenal syndrome: novel mutations in the OCRL1 gene and correlation of direct DNA diagnosis with ocular examination. *Mol. Genet. Metab.* 69: 213-222.
7. Dressman, M.A., et al. 2000. OCRL1, a PtdIns(4,5)P₂ 5-phosphatase, is localized to the *trans*-Golgi network of fibroblasts and epithelial cells. *J. Histochem. Cytochem.* 48: 179-190.

CHROMOSOMAL LOCATION

Genetic locus: *Ocrl* (mouse) mapping to X A4.

PRODUCT

OCRL 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see OCRL shRNA Plasmid (m): sc-39074-SH and OCRL shRNA (m) Lentiviral Particles: sc-39074-V as alternate gene silencing products.

For independent verification of OCRL (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-39074A, sc-39074B and sc-39074C.

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

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

OCRL (C-2): sc-393577 is recommended as a control antibody for monitoring of OCRL 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 Marker[™] 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 OCRL gene expression knockdown using RT-PCR Primer: OCRL (m)-PR: sc-39074-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.