

Occludin siRNA (h2): sc-44316

BACKGROUND

Occludin is an integral membrane protein closely associated with the tight junctions of epithelial and endothelial cells. Occludin is a tetraspan integral membrane protein in epithelial and endothelial tight junction (TJ) structures that can contain two extracellular loops. The protein exists in a variety of phosphorylated forms. Phosphorylation is involved in regulating both the localization and the function of Occludin. Expression of Occludin is upregulated by poly-unsaturated fatty acids, increasing transendothelial cell resistance and reducing cellular permeability to large molecules. The level of Occludin varies greatly depending on tissue; in brain tissue, Occludin is highly expressed at cell-cell contact sites. Non-neural tissues show lower expression and discontinuous distribution. Upregulation of epithelial Occludin may play a role in enhancing paracellular permeability and be related to the damage to the tight junction.

REFERENCES

1. Mitic, L.L., et al. 1998. Molecular architecture of tight junctions. *Annu. Rev. Physiol.* 60: 121-142.
2. Furuse, M., et al. 1993. Occludin: a novel integral membrane protein localizing at tight junctions. *J. Cell Biol.* 123: 1777-1788.
3. Tsukita, S., et al. 1996. Molecular dissection of tight junctions. *Cell Struct. Funct.* 21: 381-385.
4. Sakakibara, A., et al. 1997. Possible involvement of phosphorylation of Occludin in tight junction formation. *J. Cell Biol.* 137: 1393-1401.
5. Wong, V. 1997. Phosphorylation of Occludin correlates with Occludin localization and function at the tight junction. *Am. J. Physiol.* 273: 1859-1867.
6. Jiang, W.G., et al. 1998. Regulation of tight junction permeability and Occludin expression by poly-unsaturated fatty acids. *Biochem. Biophys. Res. Commun.* 244: 414-420.
7. Hirase, T., et al. 1997. Occludin as a possible determinant of tight junction permeability in endothelial cells. *J. Cell Sci.* 110: 1603-1613.

CHROMOSOMAL LOCATION

Genetic locus: OCLN (human) mapping to 5q13.2.

PRODUCT

Occludin siRNA (h2) 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 Occludin shRNA Plasmid (h2): sc-44316-SH and Occludin shRNA (h2) Lentiviral Particles: sc-44316-V as alternate gene silencing products.

For independent verification of Occludin (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44316A, sc-44316B and sc-44316C.

PROTOCOLS

See our web site at www.scbt.com or our catalog 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 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

Occludin siRNA (h2) is recommended for the inhibition of Occludin 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

Occludin (E-5): sc-133256 is recommended as a control antibody for monitoring of 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 Occludin gene expression knockdown using RT-PCR Primer: Occludin (h2)-PR: sc-44316-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.