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Occludin shRNA (h2) Lentiviral Particles: sc-44316-V

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

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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.
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CHROMOSOMAL LOCATION

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

PRODUCT

Occludin shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Occludin siRNA (h2): sc-44316 and Occludin shRNA Plasmid (h2): sc-44316-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

Occludin shRNA (h2) Lentiviral Particles is recommended for the inhibition of Occludin expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

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 $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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