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# ZO-1 shRNA (h2) Lentiviral Particles: sc-44288-V

## BACKGROUND

Tight junctions are complexes of proteins that create intercellular boundaries between the plasma membrane domains of epithelial and endothelial cells. Many of the tight junction-associated proteins are members of the membrane-associated guanylate kinase (MAGUK) family and include occludin, ZO-1, ZO-2 and ZO-3. These proteins are thought to have both structural and signaling roles, and are characteristically defined by three protein-protein interaction modules: the PDZ domain, the SH3 domain and the guanylate kinase (GuK) domain. ZO-1 forms complexes with either ZO-2 or ZO-3. In addition, these proteins can also associate with claudin, Occludin and F-Actin, at tight junction stands, where they provide a linkage between the Actin cytoskeleton and the tight junction. ZO-1 expression is significantly reduced in many breast cancer lines. ZO-2 and ZO-3 are ubiquitously expressed within epithelial tight junctions and, unlike ZO-1, which is also expressed at cell junctions of cardiac myocytes, ZO-2 is not expressed in nonepithelial tissue.

## REFERENCES

1. Furuse, M., et al. 1994. Direct association of Occludin with ZO-1 and its possible involvement in the localization of Occludin at tight junctions. *J. Cell Biol.* 127: 1617-1626.
2. Anderson, J.M. 1996. Cell signalling: MAGUK magic. *Curr. Biol.* 6: 382-384.
3. Hoover, K.B., et al. 1998. Loss of the tight junction MAGUK ZO-1 in breast cancer: relationship to glandular differentiation and loss of heterozygosity. *Am. J. Pathol.* 153: 1767-1773.
4. Haskins, J., et al. 1998. ZO-3, a novel member of the MAGUK protein family found at the tight junction, interacts with ZO-1 and occludin. *J. Cell Biol.* 141: 199-208.
5. Itoh, M., et al. 1999. Direct binding of three tight junction-associated MAGUKs, ZO-1, ZO-2, and ZO-3, with the COOH termini of claudins. *J. Cell Biol.* 147: 1351-1363.
6. Itoh, M., et al. 1999. Characterization of ZO-2 as a MAGUK family member associated with tight as well as adherens junctions with a binding affinity to Occludin and  $\alpha$  catenin. *J. Biol. Chem.* 274: 5981-5986.

## CHROMOSOMAL LOCATION

Genetic locus: TJP1 (human) mapping to 15q13.1.

## PRODUCT

ZO-1 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  $\mu$ l frozen stock containing  $1.0 \times 10^6$  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 ZO-1 siRNA (h2): sc-44288 and ZO-1 shRNA Plasmid (h2): sc-44288-SH as alternate gene silencing products.

## STORAGE

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

## APPLICATIONS

ZO-1 shRNA (h2) Lentiviral Particles is recommended for the inhibition of ZO-1 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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

ZO-1 (H-300): sc-10804 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor gene expression knockdown using RT-PCR Primer: ZO-1 (h2)-PR: sc-44288-PR (20  $\mu$ l, 540 bp). 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.