

ZO-1 siRNA (h2): sc-44288

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

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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. Furuse, M., et al. 1999. Manner of interaction of heterogeneous claudin species within and between tight junction strands. *J. Cell Biol.* 147: 891-903.
6. 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.
7. 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 a catenin. *J. Biol. Chem.* 274: 5981-5986.
8. Wittchen, E.S., et al. 1999. Protein interactions at the tight junction. Actin has multiple binding partners, and ZO-1 forms independent complexes with ZO-2 and ZO-3. *J. Biol. Chem.* 274: 35179-35185.

CHROMOSOMAL LOCATION

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

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.

PRODUCT

ZO-1 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 ZO-1 shRNA Plasmid (h2): sc-44288-SH and ZO-1 shRNA (h2) Lentiviral Particles: sc-44288-V as alternate gene silencing products.

For independent verification of ZO-1 (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-44288A, sc-44288B and sc-44288C.

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

ZO-1 siRNA (h2) is recommended for the inhibition of ZO-1 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

ZO-1 (R40.76): sc-33725 is recommended as a control antibody for monitoring of ZO-1 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ZO-1 gene expression knockdown using RT-PCR Primer: ZO-1 (h2)-PR: sc-44288-PR (20 μ l, 540 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.