

ZO-2 siRNA (m): sc-29926

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 strands, 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. 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.
4. 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.
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.

CHROMOSOMAL LOCATION

Genetic locus: Tjp2 (mouse) mapping to 19 B.

PRODUCT

ZO-2 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 ZO-2 shRNA Plasmid (m): sc-29926-SH and ZO-2 shRNA (m) Lentiviral Particles: sc-29926-V as alternate gene silencing products.

For independent verification of ZO-2 (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-29926A, sc-29926B and sc-29926C.

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-2 siRNA (m) is recommended for the inhibition of ZO-2 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

ZO-2 (E-3): sc-515115 is recommended as a control antibody for monitoring of ZO-2 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 ZO-2 gene expression knockdown using RT-PCR Primer: ZO-2 (m)-PR: sc-29926-PR (20 μ l, 432 bp). 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.