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Cingulin siRNA (h): sc-45677

BACKGROUND

Diffusion of solutes is prevented across certain barriers by the formation of tight junction seals. Occludin and Cingulin interact with other proteins to direct the formation and regulation of tight junctions. Cingulin, a protein component of the submembrane plaque of tight junctions (TJ), contains globular and coiled-coil domains and interacts *in vitro* with several TJ and cytoskeletal proteins, including the PDZ protein ZO-1. Cingulin binding has also been shown to inhibit RhoA activation and signaling with increased Cingulin expression in confluent cells, causing downregulation of RhoA by inhibiting GEF-H1/Lfc.

REFERENCES

1. D'Atri, F., et al. 2001. Cingulin interacts with F-Actin *in vitro*. FEBS Lett. 507: 21-24.
2. D'Atri, F., et al. 2002. Evidence for a functional interaction between Cingulin and ZO-1 in cultured cells. J. Biol. Chem. 277: 27757-27764?
3. Bordin, M., et al. 2004. Histone deacetylase inhibitors up-regulate the expression of tight junction proteins. Mol. Cancer Res. 2: 692-701.
4. Guillemot, L., et al. 2004. Disruption of the cingulin gene does not prevent tight junction formation but alters gene expression. J. Cell Sci. 117: 5245-5256.
5. Umeda, K., et al. 2004. Establishment and characterization of cultured epithelial cells lacking expression of ZO-1. J. Biol. Chem. 279: 44785-44794.
6. Ajijaz, S., et al. 2005. Binding of GEF-H1 to the tight junction-associated adaptor Cingulin results in inhibition of Rho signaling and G₁/S phase transition. Dev. Cell 8: 777-786.

CHROMOSOMAL LOCATION

Genetic locus: CGN (human) mapping to 1q21.3.

PRODUCT

Cingulin siRNA (h) 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 Cingulin shRNA Plasmid (h): sc-45677-SH and Cingulin shRNA (h) Lentiviral Particles: sc-45677-V as alternate gene silencing products.

For independent verification of Cingulin (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-45677A, sc-45677B and sc-45677C.

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

Cingulin siRNA (h) is recommended for the inhibition of Cingulin 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

Cingulin (G-6): sc-365264 is recommended as a control antibody for monitoring of Cingulin 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 Cingulin gene expression knockdown using RT-PCR Primer: Cingulin (h)-PR: sc-45677-PR (20 μ l, 593 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.