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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

Cingulin shRNA (h) Lentiviral Particles: sc-45677-V

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

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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. Aijaz, 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 shRNA (h) 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 Cingulin siRNA (h): sc-45677 and Cingulin shRNA Plasmid (h): sc-45677-SH as alternate gene silencing products.

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.

APPLICATIONS

Cingulin shRNA (h) Lentiviral Particles is recommended for the inhibition of Cingulin 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

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 (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 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.

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.

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.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.