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ZnT-3 shRNA (h) Lentiviral Particles: sc-45964-V

BACKGROUND

Zinc, an essential element required for cell proliferation and differentiation, plays a role in a diverse array of cellular functions, including acting as a co-factor for numerous enzymes and transcription factors and as a neuro-regulator. The zinc transporter (ZnT) family regulates the supply of zinc within cells, and its members are characterized by containing six membrane-spanning domains, a large histidine-rich intracellular loop and a C-terminal tail. ZnT-3 mediates the uptake of zinc into vesicles in brain and testis.

REFERENCES

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2. Palmiter, R.D., Cole, T.B., Quaife, C.J. and Findley, S.D. 1996. ZnT-3, a putative transporter of zinc into synaptic vesicles. *Proc. Natl. Acad. Sci. USA* 93: 14934-14939.
3. Wenzel, H.J., Cole, T.B., Born, D.E., Schwartzkroin, P.A. and Palmiter, R.D. 1997. Ultrastructural localization of zinc transporter-3 (ZnT-3) to synaptic vesicle membranes within mossy fiber boutons in the hippocampus of mouse and monkey. *Proc. Natl. Acad. Sci. USA* 94: 12676-12681.
4. McMahon, R.J. and Cousins, R.J. 1998. Mammalian zinc transporters. *J. Nutr.* 128: 667-670.
5. Lee, J.Y., Cole, T.B., Palmiter, R.D. and Koh, J.Y. 2000. Accumulation of zinc in degenerating hippocampal neurons of ZnT3-null mice after seizures: evidence against synaptic vesicle origin. *J. Neurosci.* 20: RC79.

CHROMOSOMAL LOCATION

Genetic locus: SLC30A3 (human) mapping to 2p23.3.

PRODUCT

ZnT-3 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 ZnT-3 siRNA (h): sc-45964 and ZnT-3 shRNA Plasmid (h): sc-45964-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

ZnT-3 shRNA (h) Lentiviral Particles is recommended for the inhibition of ZnT-3 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

ZnT-3 (G-14): sc-27508 is recommended as a control antibody for monitoring of ZnT-3 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 ZnT-3 gene expression knockdown using RT-PCR Primer: ZnT-3 (h)-PR: sc-45964-PR (20 μ l). 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.