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- Trockeneiszuschlag
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SANTA CRUZ BIOTECHNOLOGY, INC.

TRPM4 shRNA (h) Lentiviral Particles: sc-45439-V

BACKGROUND

Transient receptor potential ion channels (TRPCs) are a superfamily of six transmembrane segment-spanning, gated cation channels. TRPC subtypes mediate store-operated Ca^{2+} entry, a process involving Ca^{2+} influx and replenishment of Ca^{2+} stores formerly emptied through the action of inositol 1,4,5-trisphospate production and other Ca^{2+} mobilizing agents. TRP ion channels influence calcium-depletion induced calcium influx processes in response to chemo-, mechano- and osmoregulatory events. TRPM4 is a transient receptor potential channel with an intrinsic voltage-sensing mechanism. Voltage dependence of TRPM4 may be functionally important, especially in excitable tissues generating plateau-like or bursting action potentials. TRPM4-mediated depolarization modulates Ca^{2+} oscillations, with downstream effects on cytokine production in T lymphocytes.

REFERENCES

1. Hoth, M., et al. 1997. Mitochondrial regulation of store-operated calcium signaling in T lymphocytes. J. Cell. Biol. 137: 633-648.
2. Plant, T.D., et al. 2003. TRPC4 and TRPC5: receptor-operated Ca^{2+}-permeable nonselective cation channels. Cell Calcium 33: 441-450.
3. Nilius, B., et al. 2003. Voltage dependence of the Ca^{2+}-activated cation channel TRPM4. J. Biol. Chem. 278: 30813-30820.
4. Launay, P., et al. 2004. TRPM4 regulates calcium oscillations after T cell activation. Science 306: 1374-1377.

CHROMOSOMAL LOCATION

Genetic locus: TRPM4 (human) mapping to 19q13.33.

PRODUCT

TRPM4 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 µl 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 TRPM4 siRNA (h): sc-45439 and TRPM4 shRNA Plasmid (h): sc-45439-SH as alternate gene silencing products.

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.

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

TRPM4 shRNA (h) Lentiviral Particles is recommended for the inhibition of TRPM4 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl 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

TRPM4 (G-20): sc-27540 is recommended as a control antibody for monitoring of TRPM4 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor TRPM4 gene expression knockdown using RT-PCR Primer: TRPM4 (h)-PR: sc-45439-PR (20 µl, 521 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.

PROTOCOLS

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