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TREM-2 shRNA (m) Lentiviral Particles: sc-45369-V

BACKGROUND

Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOS), known as "Nasu-Hakola disease," is a recessively inherited disease where individuals display early-onset progressive dementia and bone cysts, which leads to death. Mutations in TYROBP (DAP12), which codes for a membrane receptor component in natural-killer and myeloid cells and mutations in triggering receptor expressed on myeloid cells-2 (TREM-2), correlate well to the pathology of PLOS. TREM-2 is a cell surface receptor on human monocyte-derived dendritic cells that forms a receptor signaling complex with DAP12 and triggers activation of the immune response in macrophages and dendritic cells (DC). The TREM-2/DAP12 complex is a molecular promoter of upregulation of C-C chemokine receptor 7, partial DC maturation, and DC survival through activation of protein tyrosine kinases and extracellular signal-regulated kinase. The human chronic inflammatory TREM-2 gene maps to chromosome 6p21.1 and encodes a 230 amino acid protein.

REFERENCES

1. Bouchon, A., et al. 2001. A DAP12-mediated pathway regulates expression of C-C chemokine receptor 7 and maturation of human dendritic cells. *J. Exp. Med.* 194: 1111-1122.
2. Daws, M.R., et al. 2001. Cloning and characterization of a novel mouse myeloid DAP12-associated receptor family. *Eur. J. Immunol.* 31: 783-791.
3. Paloneva, J., et al. 2002. Mutations in two genes encoding different subunits of a receptor signaling complex result in an identical disease phenotype. *Am. J. Hum. Gen.* 71: 656-662.
4. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 605086. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

CHROMOSOMAL LOCATION

Genetic locus: Trem2 (mouse) mapping to 17 C.

PRODUCT

TREM-2 shRNA (m) 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 TREM-2 siRNA (m): sc-45369 and TREM-2 shRNA Plasmid (m): sc-45369-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.

APPLICATIONS

TREM-2 shRNA (m) Lentiviral Particles is recommended for the inhibition of TREM-2 expression in mouse 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

TREM-2 (M-227): sc-48765 is recommended as a control antibody for monitoring of TREM-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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor TREM-2 gene expression knockdown using RT-PCR Primer: TREM-2 (m)-PR: sc-45369-PR (20 µl, 497 bp). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.

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.

PROTOCOLS

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