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Mast Cell Tryptase shRNA (m) Lentiviral Particles: sc-44922-V

BACKGROUND

Mast Cells are connective tissue cells derived from blood-forming tissues that line arterial walls and secrete substances, which mediate inflammatory and immune responses. Mast Cell chymase, known as CMA1, is a major secreted serine protease that is involved in vasoactive peptide generation, extracellular matrix degradation and regulation of gland secretion. The human chymase gene, which maps to human chromosome 14q11.2, encodes a proenzyme with a 19-amino acid signal peptide, an acidic 2-amino acid propeptide and a 226-amino acid catalytic domain. Tryptases comprise a family of trypsin-like serine proteases that are enzymatically active as heparin-stabilized tetramers. There are four functional genes for tryptase: α I, β I, β II and γ I, which map to human chromosome 16p13.3, with β tryptases representing the main isoenzymes expressed in mast cells. Mast Cell proteases are a family of rodent protein homologs to human tryptases that are specifically expressed in mast cells and may serve as highly specific markers in the analysis of Mast Cell heterogeneity, differentiation and function.

REFERENCES

- Huang, R.Y., et al. 1991. Cloning and structural analysis of MMCP-1, MMCP-4 and MMCP-5, three mouse mast cell-specific serine proteases. *Eur. J. Immunol.* 21: 1611-1621.
- Caughey, G.H., et al. 1991. Structure, chromosomal assignment and deduced amino acid sequence of a human gene for mast cell chymase. *J. Biol. Chem.* 266: 12956-12963.
- Caughey, G.H., et al. 1993. The human mast cell chymase gene (CMA1): mapping to the cathepsin G/granzyme gene cluster and lineage-restricted expression. *Genomics* 15: 614-620.

CHROMOSOMAL LOCATION

Genetic locus: Tpsb2 (mouse) mapping to 17 A3.3.

PRODUCT

Mast Cell Tryptase 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 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 Mast Cell Tryptase siRNA (m): sc-44922 and Mast Cell Tryptase shRNA Plasmid (m): sc-44922-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.

PROTOCOLS

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

APPLICATIONS

Mast Cell Tryptase shRNA (m) Lentiviral Particles is recommended for the inhibition of Mast Cell Tryptase expression in mouse 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

Mast Cell Tryptase (G-12): sc-32474 is recommended as a control antibody for monitoring of Mast Cell Tryptase 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 Mast Cell Tryptase gene expression knockdown using RT-PCR Primer: Mast Cell Tryptase (m)-PR: sc-44922-PR (20 μ l, 549 bp). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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.