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cathepsin R shRNA (m) Lentiviral Particles: sc-44642-V

BACKGROUND

The cathepsin family of proteolytic enzymes contains several diverse classes of proteases. The cysteine protease class comprises cathepsins B, L, H, K, S and O. The aspartyl protease class is composed of cathepsins D and E. Cathepsin G is in the serine protease class. Most cathepsins are lysosomal and each is involved in cellular metabolism, participating in various events such as peptide biosynthesis and protein degradation. Cathepsin G expression is restricted to bone marrow and early myeloid cells. RT-PCR analysis indicates that cathepsin R is only expressed in placenta and thus is a new member of the emerging family of cathepsins whose expression is regulated during mouse embryonic development. The mouse placentally expressed cathepsin genes, cathepsin M, P, Q, R, 1, 2, 3 and 6, are located in a tight cluster on chromosome 13. Cathepsin M, Q and R are found in mice and rats, but homologs of these genes are not found in humans or rabbits.

REFERENCES

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2. Ishidoh, K., et al. 1987. Molecular cloning and sequencing of cDNA for rat cathepsin H. Homology in pro-peptide regions of cysteine proteases. *FEBS Lett.* 226: 33-37.
3. Redecker, B., et al. 1991. Molecular organization of the human cathepsin D gene. *DNA Cell Biol.* 10: 423-431.
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5. Heusel, J.W., et al. 1993. Molecular cloning, chromosomal location, and tissue-specific expression of the murine cathepsin G gene. *Blood* 81: 1614-1623.
6. Guenette, R.S., et al. 1994. Cathepsin B, a cysteine protease implicated in metastatic progression, is also expressed during regression of the rat prostate and mammary glands. *Eur. J. Biochem.* 226: 311-321.

CHROMOSOMAL LOCATION

Genetic locus: *Ctsr* (mouse) mapping to 13 B2.

PRODUCT

cathepsin R 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 cathepsin R siRNA (m): sc-44642 and cathepsin R shRNA Plasmid (m): sc-44642-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

cathepsin R shRNA (m) Lentiviral Particles is recommended for the inhibition of cathepsin R 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

cathepsin D (C-5): sc-377124 is recommended as a control antibody for monitoring of cathepsin R 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 cathepsin R gene expression knockdown using RT-PCR Primer: cathepsin R (m)-PR: sc-44642-PR (20 μ l). 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.

PROTOCOLS

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