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Diagnostik & molekulare Diagnostik



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cathepsin V shRNA (h) Lentiviral Particles: sc-44526-V



The Power to Ouestion

BACKGROUND

Cathepsin V (CTSU, CTSV, CATL2, cathepsin U, cathepsin L2) is a lysosomal cysteine proteinase that influences corneal physiology, and mediates degradation of invariant chain in human thymus. Cathepsin V is present in corneal epithelium, activated macrophages, and colorectal and breast carcinomas. A 1.8-kb mRNA to Cathepsin V is present in normal human thymus and testis. Cathepsin V is a member of the peptidase C1 family. Cysteine proteinases are synthesized as preproenzymes, which are processed to the corresponding proenzymes. The proenzymes are either targeted to the lysosome or continue along the cellular secretory route.

REFERENCES

- Adachi, W., et al. 1998. Isolation and characterization of human cathepsin V: a major proteinase in corneal epithelium. Invest. Ophthalmol. Vis. Sci. 39: 1789-1796.
- Santamaria, I., et al. 1998. Cathepsin L2, a novel human cysteine proteinase produced by breast and colorectal carcinomas. Cancer Res. 58: 1624-1630.
- Bromme, D., et al. 1999. Human cathepsin V functional expression, tissue distribution, electrostatic surface potential, enzymatic characterization and chromosomal localization. Biochemistry 38: 2377-2385.
- Tolosa, E., et al. 2003. Cathepsin V is involved in the degradation of invariant chain in human thymus and is overexpressed in myasthenia gravis. J. Clin. Invest. 112: 517-526.

CHROMOSOMAL LOCATION

Genetic locus: CTSL2 (human) mapping to 9q22.33.

PRODUCT

cathepsin V 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 x 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 V siRNA (h): sc-44526 and cathepsin V shRNA Plasmid (h): sc-44526-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

cathepsin V siRNA (h) is recommended for the inhibition of cathepsin V expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0 x 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 V (CV55-3G11): sc-32798 is recommended as a control antibody for monitoring of cathepsin V 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) Immunofluo-rescence: 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 V gene expression knockdown using RT-PCR Primer: cathepsin V (h)-PR: sc-44526-PR (20 μ I, 461 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.

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.

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