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cystatin S/SA/SN shRNA (h) Lentiviral Particles: sc-44521-V

BACKGROUND

The cystatin superfamily is a well-established family of cysteine protease inhibitors. Cystatins A and B (type 1) are mainly intracellular; cystatins C, D, E/M, F, G, S, SN and SA cystatins are extracellular (type 2); and the kininogens are type 3 cystatins which are intravascular proteins. All true cystatins inhibit cysteine peptidases of the papain family, such as cathepsins, and some also inhibit legumain family enzymes. Cystatin SA, cystatin S and cystatin SN are found primarily in saliva. Cystatin S and SN can also be expressed in tears, urine and seminal fluid. Cystatin C is a related protein which is expressed in brain, thymus, ovary, epididymis and vas deferens. Cystatin D protects against proteinases in the oral cavity, while Cystatin E/M and F moderate the inhibition of cathepsin proteins. The fetuins, part of the cystatin superfamily, are secretable proteins that influence osteogenesis and bone resorption, regulation of the Insulin and hepatocyte growth factor receptors and the response to systemic inflammation. High molecular weight kininogen (Kininogen HC) and low molecular weight kininogen (Kininogen LC) have varied roles, though they both inhibit the thrombin- and plasmin-induced aggregation of thrombocytes.

REFERENCES

1. Saitoh, E., et al. 1988. Cystatin superfamily. Evidence that family II cystatin genes are evolutionarily related to family III cystatin genes. *Biol. Chem. Hoppe-Seyler* 369: 191-197.
2. Online Mendelian Inheritance in Man, OMIM[™]. 1999. Johns Hopkins University, Baltimore, MD. MIM Number: 604312: World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
3. Nishio, C., et al. 2000. Involvement of cystatin C in oxidative stress-induced apoptosis of cultured rat CNS neurons. *Brain Res.* 873: 252-262.
4. Manoury, B., et al. 2001. Bm-CPI-2, a cystatin homolog secreted by the filarial parasite *Brugia malayi*, inhibits class II MHC-restricted antigen processing. *Curr. Biol.* 11: 447-451.

CHROMOSOMAL LOCATION

Genetic locus: CST4/CST2/CST1 (human) mapping to 20p11.21.

PRODUCT

cystatin S/SA/SN shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 2 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 cystatin S/SA/SN siRNA (h): sc-44521 and cystatin S/SA/SN shRNA Plasmid (h): sc-44521-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

cystatin S/SA/SN shRNA (h) Lentiviral Particles is recommended for the inhibition of cystatin S/SA/SN expression in human 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

cystatin S/SA/SN (Z8983): sc-73884 is recommended as a control antibody for monitoring of cystatin S/SA/SN 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 cystatin S/SA/SN gene expression knockdown using RT-PCR Primer: cystatin S/SA/SN (h)-PR: sc-44521-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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