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cystatin B shRNA (m) Lentiviral Particles: sc-44743-V

BACKGROUND

Cystatin A (also designated STF1, STFA, stefin A or cystatin AS) and Cystatin B (also designated PME, CST6, STFB, CPI-B, stefin B and liver thiol proteinase inhibitor) are thiol protease inhibitors that form complexes with papain and the cathepsins B, H and L. Cystatin A, a cytoplasmic protein, is one of the precursor proteins of the cornified cell envelope in keratinocytes and plays a role in epidermal development and maintenance. Cystatin >B protects against intracellular proteases leaking out of lysosomes and is primarily expressed in heart, liver and kidney.

REFERENCES

1. Ritonja, A., et al. 1985. Amino acid sequence of the intracellular cysteine proteinase inhibitor cystatin B from human liver. *Biochem. Biophys. Res. Commun.* 131: 1187-1192.
2. Jerala, R., et al. 1988. Cloning a synthetic gene for human stefin B and its expression in *E. coli*. *FEBS Lett.* 239: 41-44.
3. Pennacchio, L.A., et al. 1996. Mutations in the gene encoding cystatin B in progressive myoclonus epilepsy (EPM1). *Science* 271: 1731-1734.
4. Kos, J., et al. 1998. Cysteine proteinases and their endogenous inhibitors: target proteins for prognosis, diagnosis and therapy in cancer (review). *Oncol. Rep.* 5: 1349-1361.
5. Takahashi, H., et al. 1998. Structure and transcriptional regulation of the human cystatin A gene. The 12-O-tetradecanoylphorbol-13-acetate (TPA) responsive element-2 site (-272 to -278) on cystatin A gene is critical for TPA-dependent regulation. *J. Biol. Chem.* 273: 17375-17380.

CHROMOSOMAL LOCATION

Genetic locus: *Cstb* (mouse) mapping to 10 C1.

PRODUCT

cystatin B 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 cystatin B siRNA (m): sc-44743 and cystatin B shRNA Plasmid (m): sc-44743-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

cystatin B shRNA (m) Lentiviral Particles is recommended for the inhibition of cystatin B 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

cystatin B (C-16): sc-33274 is recommended as a control antibody for monitoring of cystatin B 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 cystatin B gene expression knockdown using RT-PCR Primer: cystatin B (m)-PR: sc-44743-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.