SANTA CRUZ BIOTECHNOLOGY, INC.

cystatin B siRNA (m): sc-44743

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.
6. Takahashi, H., et al. 2001. Expression of human cystatin A by keratinocytes is positively regulated via the Ras/MEKK1/MKK7/JNK signal transduction pathway but negatively regulated via the Ras/Raf-1/MEK1/ERK pathway. J. Biol. Chem. 276: 36632-36638.
7. Jenko, S., et al. 2004. Different propensity to form amyloid fibrils by two homologous proteins—human stefins A and B: searching for an explanation. Proteins 55: 417-425.

CHROMOSOMAL LOCATION

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

PRODUCT

cystatin B siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see cystatin B shRNA Plasmid (m): sc-44743-SH and cystatin B shRNA (m) Lentiviral Particles: sc-44743-V as alternate gene silencing products.

For independent verification of cystatin B (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44743A, sc-44743B and sc-44743C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

cystatin B siRNA (m) is recommended for the inhibition of cystatin B expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

cystatin B (F-5): sc-166561 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 reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

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 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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