

cystatin D siRNA (h): sc-60489

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 secreted 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. Nishio, C., et al. 2000. Involvement of cystatin C in oxidative stress-induced apoptosis of cultured rat CNS neurons. *Brain Res.* 873: 252-262.
3. 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.
4. Janowski, R., et al. 2001. Human cystatin C, an amyloidogenic protein, dimerizes through three-dimensional domain swapping. *Nat. Struct. Biol.* 8: 316-320.
5. Aras, O., et al. 2001. Cystatin C is an independent predictor of fasting and post-methionine load total homocysteine concentrations among stable renal transplant recipients. *Clin. Chem.* 47: 1263-1268.
6. Calero, M., et al. 2001. Distinct properties of wild-type and the amyloidogenic human cystatin C variant of hereditary cerebral hemorrhage with amyloidosis, Icelandic type. *J. Neurochem.* 77: 628-637.

CHROMOSOMAL LOCATION

Genetic locus: CST5 (human) mapping to 20p11.21.

PRODUCT

cystatin D siRNA (h) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see cystatin D shRNA Plasmid (h): sc-60489-SH and cystatin D shRNA (h) Lentiviral Particles: sc-60489-V as alternate gene silencing products.

For independent verification of cystatin D (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-60489A, sc-60489B and sc-60489C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCL, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

cystatin D siRNA (h) is recommended for the inhibition of cystatin D expression in human 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 μ M in 66 μ l. 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 D (F-3): sc-398553 is recommended as a control antibody for monitoring of cystatin D 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 D gene expression knockdown using RT-PCR Primer: cystatin D (h)-PR: sc-60489-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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

PROTOCOLS

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