

cystatin C siRNA (h): sc-43714

BACKGROUND

Cystatin C is a cysteine (thiol) protease inhibitor that belongs to the type II cystatin gene superfamily and is the most abundant extracellular inhibitor of cysteine proteases. Cystatin C is a constitutively secreted, amyloidogenic protein, which forms a two-fold symmetric dimer and modulates both cysteine protease activity and the expression of class II MHC molecules. Expression of cystatin C is an indicator of kidney function and glomerular filtration rate. Mutations in the cystatin C gene can lead to protein aggregates, which are implicated in hereditary amyloid angiopathy (HCAA) and cerebral hemorrhage. Although both wildtype and mutant cystatin C are capable of forming concentration dependent inactive dimers, mutant cystatin C dimerizes at lower concentrations and is more susceptible to serine proteases, which may facilitate aggregation. In neuronal cells, oxidative stress stimulates expression of cystatin C, which may positively regulate apoptosis.

REFERENCES

1. 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/>
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. Janowski, R., et al. 2001. Human cystatin C, an amyloidogenic protein, dimerizes through three-dimensional domain swapping. *Nat. Struct. Biol.* 8: 316-320.
4. 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.
5. 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.
6. 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: CST3 (human) mapping to 20p11.21.

PRODUCT

cystatin C 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 C shRNA Plasmid (h): sc-43714-SH and cystatin C shRNA (h) Lentiviral Particles: sc-43714-V as alternate gene silencing products.

For independent verification of cystatin C (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-43714A, sc-43714B and sc-43714C.

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 C siRNA (h) is recommended for the inhibition of cystatin C 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 C (A-10): sc-515732 is recommended as a control antibody for monitoring of cystatin C 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 C gene expression knockdown using RT-PCR Primer: cystatin C (h)-PR: sc-43714-PR (20 μ l, 464 bp). 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.