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### SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

[mail@szabo-scandic.com](mailto:mail@szabo-scandic.com)

[www.szabo-scandic.com](http://www.szabo-scandic.com)

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

# cystatin C shRNA (h) Lentiviral Particles: sc-43714-V

## 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 wild-type 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. 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.

## CHROMOSOMAL LOCATION

Genetic locus: CST3 (human) mapping to 20p11.21.

## PRODUCT

cystatin C shRNA (h) 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 µl frozen stock containing  $1.0 \times 10^6$  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 C siRNA (h): sc-43714 and cystatin C shRNA Plasmid (h): sc-43714-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 C shRNA (h) Lentiviral Particles is recommended for the inhibition of cystatin C expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing  $1.0 \times 10^6$  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 C (C-27): sc-73878 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 (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 C gene expression knockdown using RT-PCR Primer: cystatin C (h)-PR: sc-43714-PR (20 µl, 464 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

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