

cathepsin W siRNA (h): sc-72807

BACKGROUND

Cathepsin W (lymphopain) and cathepsin F comprise a novel subgroup of cathepsin proteases, and are phylogenetically distinct from other human cathepsins. The cathepsin W gene maps to chromosome 11q13.1 and contains ten exons with introns ranging from 81-119 bp. Cathepsin W protein is expressed specifically in CD8⁺ T lymphocytes. The expression of cathepsin W first occurs during the differentiation of thymocytes to CD8⁺ T lymphocytes, just as the thymocytes cease expression of CD4⁺ receptors. In transfected Cos-7 and HeLa cells, cathepsin W localizes within the rough endoplasmic reticulum. Cathepsin W contains a unique 21 amino acid peptide insertion between the active site histidine and asparagine residues, in addition to a distinctive 8-amino acid carboxy-terminal extension. An extended loop structure in the second or beta-sheet domain and an additional disulfide bond are two of several signature features of cathepsin W. Other features of cathepsin W include an additional cysteine, an S2 pocket and an additional residue. Cathepsin W may exist as a dimer with each monomer forming a disulfide bond.

REFERENCES

1. Linnevers, C., et al. 1997. Human cathepsin W, a putative cysteine protease predominantly expressed in CD8⁺ T-lymphocytes. *FEBS Lett.* 405: 253-259.
2. Wex, T., et al. 1998. Genomic structure, chromosomal localization, and expression of human cathepsin W. *Biochem. Biophys. Res. Commun.* 248: 255-261.
3. Wex, T., et al. 1999. Human cathepsins F and W: a new subgroup of cathepsins. *Biochem. Biophys. Res. Commun.* 259: 401-407.
4. Bhandoola, A., et al. 2000. Programming for cytotoxic effector function occurs concomitantly with CD4 extinction during CD8⁺ T cell differentiation in the thymus. *Int. Immunol.* 12: 1035-1040.
5. Brinkworth, R.L., et al. 2000. Phylogenetic relationships and theoretical model of human cathepsin W (lymphopain), a cysteine proteinase from cytotoxic T lymphocytes. *Int. J. Biochem. Cell Biol.* 32: 373-384.

CHROMOSOMAL LOCATION

Genetic locus: CTSW (human) mapping to 11q13.1.

PRODUCT

cathepsin W 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 cathepsin W shRNA Plasmid (h): sc-72807-SH and cathepsin W shRNA (h) Lentiviral Particles: sc-72807-V as alternate gene silencing products.

For independent verification of cathepsin W (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-72807A, sc-72807B and sc-72807C.

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 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

cathepsin W siRNA (h) is recommended for the inhibition of cathepsin W 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

cathepsin W (1B1): sc-32799 is recommended as a control antibody for monitoring of cathepsin W 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 cathepsin W gene expression knockdown using RT-PCR Primer: cathepsin W (h)-PR: sc-72807-PR (20 μ l). 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.