

cathepsin D siRNA (r): sc-270475

BACKGROUND

The cathepsin family of proteolytic enzymes contains several diverse classes of proteases. The cysteine protease class comprises cathepsins B, L, H, K, S, and O. The aspartyl protease class is composed of cathepsins D and E. Cathepsin G is in the serine protease class. Most cathepsins are lysosomal and each is involved in cellular metabolism, participating in various events such as peptide biosynthesis and protein degradation. Cathepsins may also cleave some protein precursors, thereby releasing regulatory peptides. The promoter region of the cathepsin D gene contains five Sp1 binding sites and four AP-2 binding sites.

REFERENCES

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2. Ishidoh, K., et al. 1987. Molecular cloning and sequencing of cDNA for rat cathepsin H. Homology in pro-peptide regions of cysteine proteases. *FEBS Lett.* 226: 33-37.
3. Redecker, B., et al. 1991. Molecular organization of the human cathepsin D gene. *DNA Cell Biol.* 10: 423-431.
4. Shi, G.P., et al. 1992. Molecular cloning and expression of human alveolar macrophage cathepsin S, an elastinolytic cysteine protease. *J. Biol. Chem.* 267: 7258-7262.
5. Heusel, J.W., et al. 1993. Molecular cloning, chromosomal location, and tissue-specific expression of the murine cathepsin G gene. *Blood* 81: 1614-1623.
6. Guenette, R.S., et al. 1994. Cathepsin B, a cysteine protease implicated in metastatic progression, is also expressed during regression of the rat prostate and mammary glands. *Eur. J. Biochem.* 226: 311-321.
7. Shi, G.P., et al. 1995. Molecular cloning of human cathepsin O, a novel endoproteinase and homologue of rabbit OC2. *FEBS Lett.* 357: 129-134.
8. Okamoto, K., et al. 1995. Isolation and sequencing of two cDNA clones encoding rat spleen cathepsin E and analysis of the activation of purified procathepsin E. *Arch. Biochem. Biophys.* 322: 103-111.

CHROMOSOMAL LOCATION

Genetic locus: Ctsd (rat) mapping to 1q41.

PRODUCT

cathepsin D siRNA (r) 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 D shRNA Plasmid (r): sc-270475-SH and cathepsin D shRNA (r) Lentiviral Particles: sc-270475-V as alternate gene silencing products.

For independent verification of cathepsin D (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270475A, sc-270475B and sc-270475C.

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 D siRNA (r) is recommended for the inhibition of cathepsin D expression in rat 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 D (D-7): sc-377299 is recommended as a control antibody for monitoring of cathepsin 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 cathepsin D gene expression knockdown using RT-PCR Primer: cathepsin D (r)-PR: sc-270475-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.