

ADAMTS-4 siRNA (h): sc-41428

BACKGROUND

ADAMTS (a disintegrin and metalloprotease with Thrombospondin motifs) protein family members contain an N-terminal propeptide domain, a metalloproteinase domain, a disintegrin-like domain, and a C-terminus that contains a varying number of thrombospondin type-1 (TSP-1) motifs. ADAMTS-4 (also known as aggrecanase-1) is an 837 amino acid, Zn-metalloprotease that mediates proteolytic degradation of Aggrecan, a major component of cartilage. Aggrecan swells and hydrates the collagen fibril meshwork in cartilage, which confers compressibility and resilience. Degradation of Aggrecan is a factor that contributes to erosion of articular cartilage in arthritic diseases. Traditional matrix metalloproteinases (MMPs) cleave Aggrecan at Asn 341-Phe 342 whereas ADAMTS-4 cleaves at Glu 373-Ala 374. Inhibitors tailored to both MMPs and ADAMTSs may hinder the rate of cartilage degradation in arthritic individuals.

REFERENCES

1. Tang, B.L., et al. 1999. ADAMTS: a novel family of proteases with an ADAM protease domain and thrombospondin 1 repeats. *FEBS Lett.* 445: 223-225.
2. Tortorella, M.D., et al. 1999. Purification and cloning of aggrecanase-1: a member of the ADAMTS family of proteins. *Science* 284: 1664-1666.
3. Tortorella, M., et al. 2000. The Thrombospondin motif of aggrecanase-1 (ADAMTS-4) is critical for aggrecan substrate recognition and cleavage. *J. Biol. Chem.* 275: 25791-25797.
4. Tortorella, M.D., et al. 2000. Sites of aggrecan cleavage by recombinant human aggrecanase-1 (ADAMTS-4). *J. Biol. Chem.* 275: 18566-18573.
5. Online Mendelian Inheritance in Man, OMIM[™]. 2000. Johns Hopkins University, Baltimore, MD. MIM Number: 603876. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

CHROMOSOMAL LOCATION

Genetic locus: ADAMTS4 (human) mapping to 1q23.3.

PRODUCT

ADAMTS-4 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 ADAMTS-4 shRNA Plasmid (h): sc-41428-SH and ADAMTS-4 shRNA (h) Lentiviral Particles: sc-41428-V as alternate gene silencing products.

For independent verification of ADAMTS-4 (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-41428A, sc-41428B and sc-41428C.

PROTOCOLS

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

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

ADAMTS-4 siRNA (h) is recommended for the inhibition of ADAMTS-4 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

ADAMTS-4 (199F2U): sc-517638 is recommended as a control antibody for monitoring of ADAMTS-4 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 ADAMTS-4 gene expression knockdown using RT-PCR Primer: ADAMTS-4 (h)-PR: sc-41428-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.