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ADAMTS-8 siRNA (h): sc-43603

BACKGROUND

ADAMTS (a disintegrin and metalloproteinase domain with Thrombospondin type 1 modules) is a family of zinc-dependent proteases that are implicated in a variety of normal and pathological conditions, including arthritis and cancer. ADAMTS protein family members contain an amino-terminal propeptide domain, a metalloproteinase domain, a disintegrin-like domain and a carboxy-terminus that contains a varying number of Thrombospondin type 1 (TSP-1) motifs. ADAMTS-8, also designated METH-2, METH-8 or FLJ41712, along with ADAMTS-1 (METH-1, C3-C5), represent a new family of proteins with metalloprotease, disintegrin and Thrombospondin domains. The spacer region and the Thrombospondin type I motifs in the carboxy-terminus of ADAMTS-8 are important for anchoring the protein to the extracellular matrix. ADAMTS-1 and ADAMTS-8 are both secreted and proteolytically processed proteins. ADAMTS-8 is highly expressed in adult and fetal lung tissue while detected at lower levels in heart, placenta, stomach, brain and kidney tissue.

REFERENCES

1. Kuno, K., et al. 1997. Molecular cloning of a gene encoding a new type of metalloproteinase-disintegrin family protein with thrombospondin motifs as an inflammation associated gene. *J. Biol. Chem.* 272: 556-562.
2. Kuno, K., et al. 1997. The exon/intron organization and chromosomal mapping of the mouse ADAMTS-1 gene encoding an ADAM family protein with TSP motifs. *Genomics* 46: 466-471.
3. Vazquez, F., et al. 1999. METH-1, a human ortholog of ADAMTS-1, and METH-2 are members of a new family of proteins with angio-inhibitory activity. *J. Biol. Chem.* 274: 23349-23357.
4. 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.
5. Tortorella, M.D., et al. 1999. Purification and cloning of aggrecanase-1: a member of the ADAMTS family of proteins. *Science* 284: 1664-1666.

CHROMOSOMAL LOCATION

Genetic locus: ADAMTS8 (human) mapping to 11q24.3.

PRODUCT

ADAMTS-8 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-8 shRNA Plasmid (h): sc-43603-SH and ADAMTS-8 shRNA (h) Lentiviral Particles: sc-43603-V as alternate gene silencing products.

For independent verification of ADAMTS-8 (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-43603A, sc-43603B and sc-43603C.

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-8 siRNA (h) is recommended for the inhibition of ADAMTS-8 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-8 (G-4): sc-514717 is recommended as a control antibody for monitoring of ADAMTS-8 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-8 gene expression knockdown using RT-PCR Primer: ADAMTS-8 (h)-PR: sc-43603-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.