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ADAMTS-8 shRNA (m) Lentiviral Particles: sc-45834-V

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 (mouse) mapping to 9 A4.

PRODUCT

ADAMTS-8 shRNA (m) 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 μ l frozen stock containing 1.0×10^6 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 ADAMTS-8 siRNA (m): sc-45834 and ADAMTS-8 shRNA Plasmid (m): sc-45834-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

ADAMTS-8 shRNA (m) Lentiviral Particles is recommended for the inhibition of ADAMTS-8 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

ADAMTS-8 (H-17): sc-5472 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ADAMTS-8 gene expression knockdown using RT-PCR Primer: ADAMTS-8 (m)-PR: sc-45834-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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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