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ATIII shRNA (h) Lentiviral Particles: sc-44839-V

BACKGROUND

The serine proteinase inhibitors (serpins) compose a superfamily of proteins with a diverse set of functions, including the control of blood coagulation, complement activation, programmed cell death and development. Serpins are secreted glycoproteins that contain a stretch of peptide that mimics a true substrate for a corresponding serine protease. Antithrombin III (ATIII), an extracellular plasma protein, is a crucial serine protease inhibitor that regulates the coagulation cascade in blood. The inhibitory activity of ATIII is amplified in the presence of heparin. ATIII inhibits Thrombin and Factors IXa, Xa and XIa. Defects in the gene SERPINC1, which encodes for ATIII, can cause ATIII deficiency, an autosomal dominant disease which is a risk factor for hereditary thrombophilia.

REFERENCES

1. Mackie, M., et al. 1978. Familial thrombosis: inherited deficiency of antithrombin III. *Br. Med. J.* 1: 136-138.
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3. Winter, J.H., et al. 1981 Transfusion studies in patients with familial antithrombin III (ATIII) deficiency: half-disappearance time of infused ATIII and influence of such infusion on platelet life-span. *Br. J. Haematol.* 49: 449-453.
4. Okajima, K., et al. 1995. ATIII Kumamoto II; a single mutation at Arg 393-His increased the affinity of ATIII for heparin. *Am. J. Hematol.* 48: 12-18.
5. Bayston, T.A., et al. 1999. Familial overexpression of β AT caused by an Asn 135-Thr substitution. *Blood* 93: 4242-4247.
6. Yeung, P.K. 2000. Transgenic ATIII (Genzyme). *IDrugs* 3: 669-673.
7. Niiya, K., et al. 2001. Two novel gene mutations in type I AT deficiency. *Int. J. Hematol.* 74: 469-472.

CHROMOSOMAL LOCATION

Genetic locus: SERPINC1 (human) mapping to 1q25.1.

PRODUCT

ATIII shRNA (h) 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 ATIII siRNA (h): sc-44839 and ATIII shRNA Plasmid (h): sc-44839-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.

PROTOCOLS

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

APPLICATIONS

ATIII shRNA (h) Lentiviral Particles is recommended for the inhibition of ATIII expression in human 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

ATIII (A-6): sc-393867 is recommended as a control antibody for monitoring of ATIII 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 goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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 goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ATIII gene expression knockdown using RT-PCR Primer: ATIII (h)-PR: sc-44839-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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.