

AAT siRNA (h): sc-40945

BACKGROUND

Cumulative damage to lung tissue by Neutrophil Elastase is responsible for the development of pulmonary emphysema, an irreversible lung disease characterized by loss of lung elasticity. α 1-antitrypsin (AAT), a 394 amino acid hepatic acute phase protein, predominantly inhibits Neutrophil Elastase. AAT is highly expressed in liver and in cultured hepatoma cells and, to a lesser extent, in macrophages. AAT is a highly polymorphic glycosylated serum protein with characteristic isoelectric-focusing patterns for most variants. The gene encoding AAT maps to a region of human chromosome 14 that includes a related serine protease inhibitor (serpin) gene which encodes corticosteroid-binding globulin. Oxidation of the methionine 358 residue in the active center of AAT results in a dramatic decrease in inhibitory activity towards elastase. AAT also has a moderate affinity for plasmin and Thrombin. AAT deficiency is associated with a 20-30 fold increased risk of precocious pulmonary emphysema.

REFERENCES

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2. Seyama, K., et al. 1991. Siiyama (Serine 53 (TCC) to Phenylalanine 53 (TTC)). A new α 1-antitrypsin-deficient variant with mutation on a predicted conserved residue of the serpin backbone. *J. Biol. Chem.* 266: 12627-12632.
3. Rosenberg, S., et al. 1994. Synthesis in yeast of a functional oxidation-resistant mutant of human α -antitrypsin. *Nature* 312: 77-80.
4. Graziadei, I., et al. 2000. A novel-binding site for the native hepatic acute-phase protein α -antitrypsin expressed on the human hepatoma cell line Hep G2 and intestinal cell line Caco 2. *Liver* 20: 240-246.
5. Rollini, P. and Fournier, R.E. 2000. Differential regulation of gene activity and chromatin structure within the human serpin gene cluster at 14q32.1 in macrophage microcell hybrids. *Nucleic Acids Res.* 28: 1767-1777.
6. Hsu, P.L., et al. 2007. α 1-antitrypsin precursor in gastric juice is a novel biomarker for gastric cancer and ulcer. *Clin. Cancer Res.* 13: 876-883.
7. Churg, A., et al. 2007. α 1-antitrypsin suppresses TNF α and MMP-12 production by cigarette smoke-stimulated macrophages. *Am. J. Respir. Cell Mol. Biol.* 37: 144-151.

CHROMOSOMAL LOCATION

Genetic locus: SERPINA1 (human) mapping to 14q32.13.

PRODUCT

AAT 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 AAT shRNA Plasmid (h): sc-40945-SH and AAT shRNA (h) Lentiviral Particles: sc-40945-V as alternate gene silencing products.

For independent verification of AAT (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-40945A, sc-40945B and sc-40945C.

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

AAT siRNA (h) is recommended for the inhibition of AAT 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

AAT (H-7): sc-166018 is recommended as a control antibody for monitoring of AAT 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 AAT gene expression knockdown using RT-PCR Primer: AAT (h)-PR: sc-40945-PR (20 μ l, 574 bp). 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.