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AATC siRNA (h): sc-45602

BACKGROUND

Aspartate aminotransferase (AAT) is an ubiquitous pyridoxal phosphate-dependent enzyme, which exists in both mitochondrial (AATM) and cytosolic (AATC) forms. The enzyme plays an important role in amino acid metabolism and in the urea and tricarboxylic acid cycles by catalyzing the conversion of L-aspartate and 2-oxoglutarate to oxaloacetate and L-glutamate. The two isoenzymes are homodimeric, but differ in expression patterns. Approximately 80% of the enzyme activity in liver is of mitochondrial origin, whereas in serum the enzyme activity is largely cytosolic. Also, AATC and AATM share nearly identical three-dimensional structures, but differ in their folding rates and in their affinity for binding to molecular chaperones, including GroEL.

REFERENCES

1. Doonan, S., et al. 1984. Structural and genetic relationships between cytosolic and mitochondrial isoenzymes. *Int. J. Biochem.* 16: 1193-1199.
2. Pol, S., et al. 1988. Nucleotide sequence and tissue distribution of the human mitochondrial aspartate aminotransferase mRNA. *Biochem. Biophys. Res. Commun.* 157: 1309-1135.
3. Panteghini, M., et al. 1990. Aspartate aminotransferase isoenzymes. *Clin. Biochem.* 23: 311-319.
4. Donate, F., et al. 1998. Opposite behavior of two isozymes when refolding in the presence of non-ionic detergents. *Protein Sci.* 7: 1811-1820.
5. Mattingly, J.R., Jr., et al. 1998. Conformation of aspartate aminotransferase isozymes folding under different conditions probed by limited proteolysis. *J. Biol. Chem.* 273: 23191-23202.
6. Online Mendelian Inheritance in Man, OMIM[™]. 1998. Johns Hopkins University, Baltimore, MD. MIM Number: 138180. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
7. SWISS-PROT/TrEMBL (P17174). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

CHROMOSOMAL LOCATION

Genetic locus: GOT1 (human) mapping to 10q24.2.

PRODUCT

AATC 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 AATC shRNA Plasmid (h): sc-45602-SH and AATC shRNA (h) Lentiviral Particles: sc-45602-V as alternate gene silencing products.

For independent verification of AATC (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-45602A, sc-45602B and sc-45602C.

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

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

AATC (H-8): sc-515641 is recommended as a control antibody for monitoring of AATC 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 AATC gene expression knockdown using RT-PCR Primer: AATC (h)-PR: sc-45602-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Bernfeld, E., et al. 2018. Phospholipase D-dependent mTORC1 activation by glutamine. *J. Biol. Chem.* 293: 16390-16401.

RESEARCH USE

For research use only, not for use in diagnostic procedures.