SANTA CRUZ BIOTECHNOLOGY, INC.

AATM siRNA (h): sc-60052

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. 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: 1309-1315.
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.

CHROMOSOMAL LOCATION

Genetic locus: GOT2 (human) mapping to 16q21.

PRODUCT

AATM 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see AATM shRNA Plasmid (h): sc-60052-SH and AATM shRNA (h) Lentiviral Particles: sc-60052-V as alternate gene silencing products.

For independent verification of AATM (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-60052A, sc-60052B and sc-60052C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

AATM siRNA (h) is recommended for the inhibition of AATM 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 µM in 66 µl. 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

AATM (E-7): sc-271702 is recommended as a control antibody for monitoring of AATM 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 AATM gene expression knockdown using RT-PCR Primer: AATM (h)-PR: sc-60052-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kang, J.H., et al. 2016. Aldehyde dehydrogenase inhibition combined with phenformin treatment reversed NSCLC through ATP depletion. Oncotarget 7: 49397-49410.
2. Lee, J.S., et al. 2016. Dual targeting of glutaminase 1 and thymidylate synthase elicits death synergistically in NSCLC. Cell Death Dis. 7: e2511.
3. 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.

PROTOCOLS

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