

AspRS siRNA (h): sc-72570

BACKGROUND

AspRS (mitochondrial aspartyl-tRNA synthetase), also known as aspartate-tRNA ligase or DARS2, is a 645 amino acid protein that belongs to the class-II aminoacyl-tRNA synthetase family. Localized to the mitochondrial matrix, AspRS exists as a homodimer and functions primarily to aminoacylate tRNA. Also, AspRS contains a 47 amino acid mitochondrial targeting signal, as well as several conserved residues involved in ATP binding, tRNA binding and aspartic acid recognition. The gene encoding AspRS maps to chromosome 1q25.1. Defects in this gene are a cause of leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL). LBSL is an autosomal recessive disease characterized by cerebellar ataxia, spasticity and dorsal column dysfunction, sometimes with a mild cognitive deficit.

REFERENCES

1. Thompson, D., et al. 2006. Molecular dynamics simulations show that bound Mg²⁺ contributes to amino acid and aminoacyl adenylate binding specificity in aspartyl-tRNA synthetase through long range electrostatic interactions. *J. Biol. Chem.* 281: 23792-23803.
2. Cardoso, A.M., et al. 2006. A non-discriminating aspartyl-tRNA synthetase from *Halobacterium salinarum*. *RNA Biol.* 3: 110-114.
3. Bernard, D., et al. 2007. Inhibition by L-aspartol adenylate of a nondiscriminating aspartyl-tRNA synthetase reveals differences between the interactions of its active site with tRNA(Asp) and tRNA(Asn). *J. Enzyme Inhib. Med. Chem.* 22: 77-82.
4. Kazakov, T., et al. 2007. Amino acid residues required for maturation, cell uptake, and processing of translation inhibitor microcin C. *J. Bacteriol.* 189: 2114-2118.
5. Scheper, G.C., et al. 2007. Mitochondrial aspartyl-tRNA synthetase deficiency causes leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation. *Nat. Genet.* 39: 534-539.

CHROMOSOMAL LOCATION

Genetic locus: DARS2 (human) mapping to 1q25.1.

PRODUCT

AspRS 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 AspRS shRNA Plasmid (h): sc-72570-SH and AspRS shRNA (h) Lentiviral Particles: sc-72570-V as alternate gene silencing products.

For independent verification of AspRS (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-72570A, sc-72570B and sc-72570C.

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 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

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

AspRS (C-6): sc-166535 is recommended as a control antibody for monitoring of AspRS 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 AspRS gene expression knockdown using RT-PCR Primer: AspRS (h)-PR: sc-72570-PR (20 μl). 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.