SANTA CRUZ BIOTECHNOLOGY, INC.

DaRS siRNA (m): sc-142877

BACKGROUND

Aminoacyl-tRNA synthetases consist of a family of enzymes that catalyze the specific aminoacylation of cognate tRNA in the initial step of ribosome-dependent protein biosynthesis. DaRS is part of a multisubunit complex of aminoacyl-tRNA synthetases and is involved in the transfer of Asp-tRNA to EF-1 α1 (elongation factor α 1). The N-terminus of DaRS in vertebrates is a newly evolved structure that contains a putative amphiphilic helix and is dissimilar between different species. The N-terminal extension acts as a switch that, when in its stretched form, reduces the rate of dissociation of Asp-tRNA from DaRS, thereby providing enough time for EF-1 α1 to interact with Asp-tRNA. This suggests that the N-terminus of DaRS plays a critical role in its catalytic function. DaRS contains two phosphorylations sites, forms homodimers and localizes to the cytoplasm.

REFERENCES

1. Lorber, B., et al. 1988. Properties of N-terminal truncated yeast aspartyl-tRNA synthetase and structural characteristics of the cleaved domain. Eur. J. Biochem. 174: 155-161.
2. Jacobo-Molina, A., et al. 1989. cDNA sequence, predicted primary structure, and evolving amphiphilic helix of human aspartyl-tRNA synthetase. J. Biol. Chem. 264: 16608-16612.
3. Mirande, M., et al. 1992. Engineering mammalian aspartyl-tRNA synthetase to probe structural features mediating its association with the multisynthetase complex. Eur. J. Biochem. 203: 459-466.
4. Escalante, C., et al. 1993. Expression of human aspartyl-tRNA synthetase in *Escherichia coli*. Functional analysis of the N-terminal putative amphiphilic helix. J. Biol. Chem. 268: 6014-6023.
5. Agou, F., et al. 1997. Aspartyl-tRNA synthetase from rat: *in vitro* functional analysis of its assembly into the multisynthetase complex. Eur. J. Biochem. 243: 259-267.

CHROMOSOMAL LOCATION

Genetic locus: Dars (mouse) mapping to 1 E4.

PRODUCT

DaRS siRNA (m) 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 DaRS shRNA Plasmid (m): sc-142877-SH and DaRS shRNA (m) Lentiviral Particles: sc-142877-V as alternate gene silencing products.

For independent verification of DaRS (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-142877A, sc-142877B and sc-142877C.

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

DaRS siRNA (m) is recommended for the inhibition of DaRS expression in mouse 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

DaRS (H-3): sc-393275 is recommended as a control antibody for monitoring of DaRS 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 DaRS gene expression knockdown using RT-PCR Primer: DaRS (m)-PR: sc-142877-PR (20 µl, 593 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.