SANTA CRUZ BIOTECHNOLOGY, INC.

AsnRS siRNA (m): sc-72566

BACKGROUND

AsnRS (cytoplasmic asparaginyl-tRNA synthetase, asparagine-tRNA ligase) is a cytoplasmic protein encoded by the human gene NARS. AsnRS belongs to the class-II aminoacyl-tRNA synthetase family. Aminoacyl tRNA synthetases (aaRS) are enzymes that catalyze the esterification of a specific amino acid or its precursor to its compatible cognate tRNA to form an aminoacyl-tRNA. The synthetase first binds ATP and the corresponding amino acid or its precursor to form an aminoacyl-adenylate and release inorganic pyrophosphate (PPi). The adenylate-aaRS complex then binds the appropriate tRNA molecule, and the amino acid is transferred from the aa-AMP to either the 2'- or 3'-OH of the last tRNA base (A76) at the 3'-end. Some synthetases also mediate a proofreading reaction to ensure high fidelity of tRNA charging; if the tRNA is found to be improperly charged, the aminoacyl-tRNA bond is hydrolyzed. AsnRS acts to attach asparagine residues to its cognate tRNA. AsnRS autoantibodies have a strong association with interstitial lung disease (ILD) and may be associated with the DR2 phenotype. In bacteria that lack AsnRS, AspRS (aspartyl-tRNA synthetase) acts to generate both Asp-tRNA (Asp) and the noncanonical, misacylated Asp-tRNA (Asn-tRNA).

REFERENCES

1. Ramirez, B.L., et al. 2006. *Brugia malayi* asparaginyl-transfer RNA synthetase induces chemotaxis of human leukocytes and activates G protein-coupled receptors CXCR1 and CXCR2. J. Infect. Dis. 193: 1164-1171.
2. Sukuru, S.C., et al. 2006. Discovering new classes of *Brugia malayi* asparaginyl-tRNA synthetase inhibitors and relating specificity to conformational change. J. Comput. Aided Mol. Des. 20: 159-178.
3. Iwasaki, W., et al. 2006. Structural basis of the water-assisted asparagine recognition by asparaginyl-tRNA synthetase. J. Mol. Biol. 360: 329-342.
4. Chuawong, P., et al. 2006. The nondiscriminating aspartyl-tRNA synthetase from *Helicobacter pylori:* anticodon-binding domain mutations that impact tRNA specificity and heterologous toxicity. Biochemistry 45: 8079-8087.

CHROMOSOMAL LOCATION

Genetic locus: Nars (mouse) mapping to 18 E1.

PRODUCT

AsnRS 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 AsnRS shRNA Plasmid (m): sc-72566-SH and AsnRS shRNA (m) Lentiviral Particles: sc-72566-V as alternate gene silencing products.

For independent verification of AsnRS (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-72566A, sc-72566B and sc-72566C.

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

AsnRS siRNA (m) is recommended for the inhibition of AsnRS 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

AsnRS (G-8): sc-271059 is recommended as a control antibody for monitoring of AsnRS 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 AsnRS gene expression knockdown using RT-PCR Primer: AsnRS (m)-PR: sc-72566-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.