

AlaRS siRNA (m): sc-72475

BACKGROUND

Aminoacyl-tRNA synthetases function to catalyze the aminoacylation of tRNAs by their corresponding amino acids, thus linking amino acids with tRNA-contained nucleotide triplets. Class II tRNA synthetases are a highly conserved subfamily of tRNA synthetases that have a catalytic domain through which they interact with the amino acid acceptor of the tRNA and a second domain through which they interact with the rest of the tRNA molecule. AlaRS (alanyl-tRNA synthetase), also known as AARS, is a 968 amino acid cytoplasmic protein that belongs to the class II subfamily of tRNA synthetases. Functioning as a monomer, AlaRS catalyzes the ATP-dependent attachment of alanine to a corresponding tRNA^{Ala}, thereby producing alanyl-tRNA^{Ala}. Defects in the gene encoding AlaRS may lead to an accumulation of misfolded proteins within the cell, ultimately leading to cell death.

REFERENCES

1. Francklyn, C. and Schimmel, P. 1989. Aminoacylation of RNA minihelices with alanine. *Nature* 337: 478-481.
2. Shiba, K., et al. 1995. Human alanyl-tRNA synthetase: conservation in evolution of catalytic core and microhelix recognition. *Biochemistry* 34: 10340-10349.
3. Nichols, R.C., et al. 1995. Localization of two human autoantigen genes by PCR screening and *in situ* hybridization—glycyl-tRNA synthetase locates to 7p15 and alanyl-tRNA synthetase locates to 16q22. *Genomics* 30: 131-132.
4. Ripmaster, T.L., et al. 1995. Wide cross-species aminoacyl-tRNA synthetase replacement *in vivo*: yeast cytoplasmic alanine enzyme replaced by human polymyositis serum antigen. *Proc. Natl. Acad. Sci. USA* 92: 4932-4936.
5. Chihade, J.W., et al. 2000. Origin of mitochondria in relation to evolutionary history of eukaryotic alanyl-tRNA synthetase. *Proc. Natl. Acad. Sci. USA* 97: 12153-12157.
6. Lovato, M.A., et al. 2001. Translocation within the acceptor helix of a major tRNA identity determinant. *EMBO J.* 20: 4846-4853.

CHROMOSOMAL LOCATION

Genetic locus: Aars (mouse) mapping to 8 E1.

PRODUCT

AlaRS 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see AlaRS shRNA Plasmid (m): sc-72475-SH and AlaRS shRNA (m) Lentiviral Particles: sc-72475-V as alternate gene silencing products.

For independent verification of AlaRS (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-72475A, sc-72475B and sc-72475C.

RESEARCH USE

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

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

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

AlaRS (A-6): sc-165990 is recommended as a control antibody for monitoring of AlaRS 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 AlaRS gene expression knockdown using RT-PCR Primer: AlaRS (m)-PR: sc-72475-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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