SANTA CRUZ BIOTECHNOLOGY, INC.

ThrRS siRNA (h): sc-76658

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. ThrRS (threonyl-tRNA synthetase), also known as TARS, is a 723 amino acid member of the class-II aminoacyl-tRNA synthetase family that catalyzes the tRNA(Thr)-threonine aminoacylation reaction. Localized to the cytoplasm, ThrRS contains a zinc-binding catalytic domain, a C terminal tRNA-binding domain and an N terminal editing domain. ThrRS has four mobile regions, three of which have a key residue that changes conformation throughout catalysis, thereby mediating the dynamics of the tRNA-amino acid reaction. The fourth mobile region contains an ordering loop which helps to close the active site once the substrate (threonine) is in place.

REFERENCES

1. Sankaranarayanan, R., et al. 1999. The structure of threonyl-tRNA synthetase-tRNA(Thr) complex enlightens its repressor activity and reveals an essential zinc ion in the active site. Cell 97: 371-381.
2. Torres-Larios, A., et al. 2003. Conformational movements and cooperativity upon amino acid, ATP and tRNA binding in threonyl-tRNA synthetase. J. Mol. Biol. 331: 201-211.
3. Ishikura, H., et al. 2003. Threonyl-tRNA synthetase of archaea: importance of the discriminator base in the aminoacylation of threonine tRNA. Nucleic Acids Symp. Ser. 83-84.
4. Ruan, B., et al. 2004. A unique hydrophobic cluster near the active site contributes to differences in borrelidin inhibition among threonyl-tRNA synthetases. J. Biol. Chem. 280: 571-577.
5. Yamasaki, Y., et al. 2006. Unusually high frequency of autoantibodies to PL7 associated with milder muscle disease in Japanese patients with polymyositis/dermatomyositis. Arthritis Rheum. 54: 2004-2009.
6. Hussain, T., et al. 2006. Posttransfer editing mechanism of a D-aminoacyl-tRNA deacylase-like domain in threonyl-tRNA synthetase from archaea. EMBO J. 25: 4152-4162.
7. Asanuma, Y., et al. 2006. Antisynthetase syndrome associated with sarcoidosis. Intern. Med. 45: 1065-1068.

CHROMOSOMAL LOCATION

Genetic locus: TARS (human) mapping to 5p13.3.

PRODUCT

ThrRS 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 ThrRS shRNA Plasmid (h): sc-76658-SH and ThrRS shRNA (h) Lentiviral Particles: sc-76658-V as alternate gene silencing products.

For independent verification of ThrRS (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-76658A, sc-76658B and sc-76658C.

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

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

ThrRS (C-3): sc-166146 is recommended as a control antibody for monitoring of ThrRS 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 ThrRS gene expression knockdown using RT-PCR Primer: ThrRS (h)-PR: sc-76658-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.

PROTOCOLS

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