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IleRS siRNA (h): sc-45473

BACKGROUND

The fidelity of protein synthesis requires efficient discrimination of amino acid substrates by aminoacyl-tRNA synthetases. Accurate discrimination of the structurally similar amino acids, valine and isoleucine, by isoleucyl-tRNA synthetase (IleRS) results, in part, from a hydrolytic editing reaction, which prevents misactivated valine from being stably joined to tRNA^{Ile}. IleRS joins Ile to tRNA(Ile) at its synthetic active site and hydrolyzes incorrectly acylated amino acids at its editing active site. A member of the aminoacyl-tRNA synthetase family, human IleRS has been identified as a target of antibodies in the autoimmune disease polymyositis.

REFERENCES

- Nichols, R.C., et al. 1995. Human isoleucyl-tRNA synthetase: sequence of the cDNA, alternative mRNA splicing, and the characteristics of an unusually long C-terminal. *Gene* 155: 299-304.
- Nordin, B.E., et al. 1999. RNA determinants for translational editing. Mischarging a minihelix substrate by a tRNA synthetase. *J. Biol. Chem.* 274: 6835-6838.
- Silvian, L.F., et al. 1999. Insights into editing from an ile-tRNA synthetase structure with tRNA^{Ile} and mupirocin. *Science* 285: 1074-1077.
- Nakama, T., et al. 2001. Structural basis for the recognition of isoleucyl-adenylate and an antibiotic, mupirocin, by isoleucyl-tRNA synthetase. *J. Biol. Chem.* 276: 47387-47393.
- Nordin, B.E., et al. 2003. Transiently misacylated tRNA is a primer for editing of misactivated adenylates by class I aminoacyl-tRNA synthetases. *Biochemistry* 42: 12989-12997.
- SWISS-PROT/TrEMBL (P41252). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

CHROMOSOMAL LOCATION

Genetic locus: IARS (human) mapping to 9q22.31.

PRODUCT

IleRS 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see IleRS shRNA Plasmid (h): sc-45473-SH and IleRS shRNA (h) Lentiviral Particles: sc-45473-V as alternate gene silencing products.

For independent verification of IleRS (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-45473A, sc-45473B and sc-45473C.

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.

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

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

IleRS (D-9): sc-271826 is recommended as a control antibody for monitoring of IleRS 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 IleRS gene expression knockdown using RT-PCR Primer: IleRS (h)-PR: sc-45473-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.