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# IleRS shRNA (m) Lentiviral Particles: sc-45474-V

## 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<sup>Ile</sup>. IleRS joins Ile to tRNA<sup>Ile</sup> 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<sup>Ile</sup> 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: lars (mouse) mapping to 13 A5.

## PRODUCT

IleRS shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see IleRS siRNA (m): sc-45474 and IleRS shRNA Plasmid (m): sc-45474-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ$  C for up to one week. Avoid repeated freeze thaw cycles.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.

## APPLICATIONS

IleRS shRNA (m) Lentiviral Particles is recommended for the inhibition of IleRS expression in mouse cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

IleRS (D-16): sc-34676 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor IleRS gene expression knockdown using RT-PCR Primer: IleRS (m)-PR: sc-45474-PR (20  $\mu$ l). Annealing temperature for the primers should be  $55-60^\circ$  C and the extension temperature should be  $68-72^\circ$  C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.