SANTA CRUZ BIOTECHNOLOGY, INC.

PheRS siRNA (h): sc-76115

BACKGROUND

The fidelity of protein synthesis requires efficient discrimination of amino acid substrates by aminoacyl-tRNA synthetases. Aminoacyl-tRNA synthetases function to catalyze the aminoacylation of tRNAs by their corresponding amino acids, thus linking amino acids with tRNA-contained nucleotide triplets. PheRS (phenylalanyl-tRNA synthetase 2, mitochondrial), also known as FARS2, is a 451 amino acid mitochondrial matrix protein that belongs to the class II aminoacyl-tRNA synthetase family. Functioning as a monomer, PheRS catalyzes the ATP-dependent conversion of L-phenylalanine and tRNA(Phe) to L-phenylalanyl-tRNA(Phe), an event that is crucial for proper translation and protein expression. The gene encoding PheRS maps to human chromosome 6, which contains 170 million base pairs and comprises nearly 6% of the human genome.

REFERENCES

1. Bullard, J.M., et al. 1999. Expression and characterization of a human mitochondrial phenylalanyl-tRNA synthetase. J. Mol. Biol. 288: 567-577.
2. Roy, H., et al. 2006. Phenylalanyl-tRNA synthetase contains a dispensable RNA-binding domain that contributes to the editing of noncognate aminoacyl-tRNA. Biochemistry 45: 9156-9162.
3. Sasaki, H.M., et al. 2006. Structural and mutational studies of the amino acid-editing domain from archaeal/eukaryal phenylalanyl-tRNA synthetase. Proc. Natl. Acad. Sci. USA 103: 14744-14749.
4. Levin, I., et al. 2007. Purification, crystallization and preliminary X-ray characterization of a human mitochondrial phenylalanyl-tRNA synthetase. Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 63: 761-764.
5. Ling, J., et al. 2007. Phenylalanyl-tRNA synthetase editing defects result in efficient mistranslation of phenylalanine codons as tyrosine. RNA 13: 1881-1886.
6. Online Mendelian Inheritance in Man, OMIM™. 2007. Johns Hopkins University, Baltimore, MD. MIM Number: 611592. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

CHROMOSOMAL LOCATION

Genetic locus: FARS2 (human) mapping to 6p25.1.

PRODUCT

PheRS 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 PheRS shRNA Plasmid (h): sc-76115-SH and PheRS shRNA (h) Lentiviral Particles: sc-76115-V as alternate gene silencing products.

For independent verification of PheRS (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-76115A, sc-76115B and sc-76115C.

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 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

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

PheRS (F-9): sc-166048 is recommended as a control antibody for monitoring of PheRS 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 goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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 goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PheRS gene expression knockdown using RT-PCR Primer: PheRS (h)-PR: sc-76115-PR (20 µl). 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 or our catalog for detailed protocols and support products.