SANTA CRUZ BIOTECHNOLOGY, INC.

PUS1 siRNA (h): sc-61417

BACKGROUND

PUS1 (Psuedouridine synthase 1) belongs to the tRNA pseudouridine synthase truA family. PUS1 functions in the conversion of uridine into pseudouridine after the nucleotide has been incorporated into RNA. It may have a functional role in tRNAs and is also thought to assist in the peptidyl transfer reaction of rRNAs. As a nucleus-resident protein, PUS1 forms a complex with RARG and the SRA1 RNA. PUS1 is widely expressed, with highest levels of expression in the brain and skeletal muscle tissues. Defects in PUS1 are a cause of myopathy with lactic acidosis and sideroblastic anemia (MLASA), also known as mitochondrial myopathy and sideroblastic anemia. MLASA is a rare autosomal recessive oxidative phosphorylation disorder specific to bone marrow and skeletal muscle. The deduced human PUS1 protein contains 348 amino acids and shares 92% sequence homology with mouse PUS1.

REFERENCES

1. Arluison, V., et al. 1998. Transfer RNA-pseudouridine synthetase Pus1 of *Saccharomyces cerevisiae* contains one atom of zinc essential for its native conformation and tRNA recognition. Biochemistry 37: 7268-7276.
2. Arluison, V., et al. 1999. RNA:pseudouridine synthetase Pus1 from *Saccharomyces cerevisiae:* oligomerization property and stoichiometry of the complex with yeast tRNA(Phe). Biochimie 81: 751-756.
3. Arluison, V., et al. 1999. Pseudouridine synthetase Pus1 of *Saccharomyces cerevisiae:* kinetic characterisation, tRNA structural requirement and real-time analysis of its complex with tRNA. J. Mol. Biol. 289: 491-502.
4. Chen, J. and Patton, J.R. 1999. Cloning and characterization of a mammalian pseudouridine synthase. RNA 5: 409-419.
5. Chen, J. and Patton, J.R. 2001. Mouse pseudouridine synthase 1: gene structure and alternative splicing of pre-mRNA. Biochem. J. 352: 465-473.
6. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 608109. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
7. Patton, J.R. and Padgett, R.W. 2003. *Caenorhabditis elegans* pseudouridine synthase 1 activity *in vivo:* tRNA is a substrate, but not U2 small nuclear RNA. Biochem. J. 372: 595-602.

CHROMOSOMAL LOCATION

Genetic locus: PUS1 (human) mapping to 12q24.33.

PRODUCT

PUS1 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 PUS1 shRNA Plasmid (h): sc-61417-SH and PUS1 shRNA (h) Lentiviral Particles: sc-61417-V as alternate gene silencing products.

For independent verification of PUS1 (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-61417A, sc-61417B and sc-61417C.

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

PUS1 siRNA (h) is recommended for the inhibition of PUS1 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

PUS1 (A-4): sc-390043 is recommended as a control antibody for monitoring of PUS1 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 PUS1 gene expression knockdown using RT-PCR Primer: PUS1 (h)-PR: sc-61417-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.