

TrpRS siRNA (h): sc-37673

BACKGROUND

Tryptophanyl-tRNA synthetases are essential enzymes that catalyze the aminoacylation of tRNA(trp) with tryptophan, an essential function of the protein synthesis machinery of the cell. Two forms of tryptophanyl-tRNA synthetase exist: a cytoplasmic form, named TrpRS (also known as WARS), and a mitochondrial form, named WARS2. In normal cells, human TrpRS exists as a full length form and as a truncated form designated mini TrpRS, which is produced by alternative splicing. Expression of mini TrpRS is highly stimulated in human cells by the addition of IFN- γ . Although both human full-length TrpRS and mini TrpRS are enzymatically active in aminoacylation, they differ in angiostatic activity. The gene encoding human TrpRS maps to chromosome 14q32.2 and the gene encoding human WARS2 maps to chromosome 1p12. The first 18 amino acids of WARS2 constitute the mitochondrial localization signal sequence.

REFERENCES

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2. Online Mendelian Inheritance in Man, OMIM[™]. 2000. Johns Hopkins University, Baltimore, MD. MIM Number: 604733. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
3. Wakasugi, K., Slike, B.M., Hood, J., Otani, A., Ewalt, K.L., Friedlander, M., Cheresch, D.A. and Schimmel, P. 2002. A human aminoacyl-tRNA synthetase as a regulator of angiogenesis. *Proc. Natl. Acad. Sci. USA* 99: 173-177.
4. Otani, A., Slike, B.M., Dorrell, M.I., Hood, J., Kinder, K., Ewalt, K.L., Cheresch, D., Schimmel, P. and Friedlander, M. 2002. A fragment of human TrpRS as a potent antagonist of ocular angiogenesis. *Proc. Natl. Acad. Sci. USA* 99: 178-183.

CHROMOSOMAL LOCATION

Genetic locus: WARS (human) mapping to 14q32.2.

PRODUCT

TrpRS 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 TrpRS shRNA Plasmid (h): sc-37673-SH and TrpRS shRNA (h) Lentiviral Particles: sc-37673-V as alternate gene silencing products.

For independent verification of TrpRS (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-37673A, sc-37673B and sc-37673C.

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

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

TrpRS (C-7): sc-374401 is recommended as a control antibody for monitoring of TrpRS 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 TrpRS gene expression knockdown using RT-PCR Primer: TrpRS (h)-PR: sc-37673-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.