SANTA CRUZ BIOTECHNOLOGY, INC.

TRNT1 siRNA (h): sc-78516

BACKGROUND

TRNT1 (tRNA nucleotidyl transferase, CCA-adding, 1), also known as CCA1, MtCCA or CGI-47, is a 434 amino acid mitochondrial protein belonging to the tRNA nucleotidyltransferase/poly(A) polymerase family. Considered a CCA-adding enzyme, TRNT1 is essential for catalyzing the addition of the CCA terminus to the 3' end of tRNA precursors, a reaction which is a fundamental prerequisite for mature tRNAs to become aminoacylated and to participate in protein biosynthesis. Existing a three isoforms produced by alternative splicing events, TRNT1 binds manganese as a cofactor and is subject to homodimerization by disulfied linkage. TRNT1 is encoded by a gene located on human chromosome 3, which houses over 1,100 genes, including a chemokine receptor (CKR) gene cluster and a variety of human cancer-related gene loci.

REFERENCES

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2. Nagaike, T., Suzuki, T., Tomari, Y., Takemoto-Hori, C., Negayama, F., Watanabe, K. and Ueda, T. 2001. Identification and characterization of mammalian mitochondrial tRNA nucleotidyltransferases. J. Biol. Chem. 276: 40041-40049.
3. Tomari, Y., Suzuki, T. and Ueda, T. 2002. tRNA recognition by CCA-adding enzyme. Nucleic Acids Res. Suppl. 2: 77-78.
4. Augustin, M.A., Reichert, A.S., Betat, H., Huber, R., Mörl, M. and Steegborn, C. 2003. Crystal structure of the human CCA-adding enzyme: insights into template-independent polymerization. J. Mol. Biol. 328: 985-994.
5. Higgins, J.J., Pucilowska, J., Lombardi, R.Q. and Rooney, J.P. 2004. Candidate genes for recessive non-syndromic mental retardation on chromosome 3p (MRT2A). Clin. Genet. 65: 496-500.
6. Xiong, Y. and Steitz, T.A. 2004. Mechanism of transfer RNA maturation by CCA-adding enzyme without using an oligonucleotide template. Nature 430: 640-645.
7. Xiong, Y. and Steitz, T.A. 2006. A story with a good ending: tRNA 3'-end maturation by CCA-adding enzymes. Curr. Opin. Struct. Biol. 16: 12-17.

CHROMOSOMAL LOCATION

Genetic locus: TRNT1 (human) mapping to 3p26.2.

PRODUCT

TRNT1 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 TRNT1 shRNA Plasmid (h): sc-78516-SH and TRNT1 shRNA (h) Lentiviral Particles: sc-78516-V as alternate gene silencing products.

For independent verification of TRNT1 (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-78516A, sc-78516B and sc-78516C.

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

TRNT1 siRNA (h) is recommended for the inhibition of TRNT1 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

TRNT1 (1G11): sc-517103 is recommended as a control antibody for monitoring of TRNT1 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 TRNT1 gene expression knockdown using RT-PCR Primer: TRNT1 (h)-PR: sc-78516-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.