SANTA CRUZ BIOTECHNOLOGY, INC.

TRMT1 siRNA (h): sc-97846

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

Transfer RNA (tRNA) modifications help regulate the efficiency of mRNA translation by maintaining the correct reading frames. N^2,N^2-dimethylguanosine tRNA methyltransferase, also known as TRMT1 or tRNA(guanine-26,N^2-N^2) methyltransferase, is a 659 amino acid enzyme that is responsible for tRNA modifications in eukaryotes. Using S-adenosyl-L-methionine as a methyl donor, TRMT1 dimethylates a single guanine residue at position 26 of tRNA. TRMT1, which was initially identified in yeast and *C. elegans,* has a 26% and 31% sequence identity to its yeast and C. elegans homologs, respectively. There are two isoforms of TRMT1 produced by alternative splicing events. The TRMT1 gene maps to chromosome 19p13.13 and mutations in this gene lead to abrogated enzyme activity and a decrease in protein levels.

REFERENCES

1. Edqvist, J., et al. 1995. Enzymatic formation of N^2,N^2-dimethylguanosine in eukaryotic tRNA: importance of the tRNA architecture. Biochimie 77: 54-61.
2. Constantinesco, F., et al. 1998. The tRNA(guanine-26,N^2-N^2) methyltransferase (Trm1) from the hyperthermophilic archaeon *Pyrococcus furiosus:* cloning, sequencing of the gene and its expression in *Escherichia coli.* Nucleic Acids Res. 26: 3753-3761.
3. Liu, J., et al. 1998. Point and deletion mutations eliminate one or both methyl group transfers catalysed by the yeast TRM1 encoded tRNA (m22G26)dimethyltransferase. Nucleic Acids Res. 26: 5102-5108.
4. Björk, G.R., et al. 1999. Transfer RNA modification: influence on translational frameshifting and metabolism. FEBS Lett. 452: 47-51.
5. Niederberger, C., et al. 1999. The tRNA N^2,N^2-dimethylguanosine-26 methyltransferase encoded by gene Trm1 increases efficiency of suppression of an ochre codon in *Schizosaccharomyces pombe.* FEBS Lett. 464: 67-70.
6. Constantinesco, F., et al. 1999. Characterisation and enzymatic properties of tRNA(guanine 26, N^2, N^2)-dimethyltransferase (Trm1p) from *Pyrococcus furiosus.* J. Mol. Biol. 291: 375-392.
7. Liu, J. and Strâby, K.B. 2000. The human tRNA($m^2_2G_{26}$)dimethyltransferase: functional expression and characterization of a cloned hTRM1 gene. Nucleic Acids Res. 28: 3445-3451.

CHROMOSOMAL LOCATION

Genetic locus: TRMT1 (human) mapping to 19p13.2.

PRODUCT

TRMT1 siRNA (h) is a pool of 2 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 TRMT1 shRNA Plasmid (h): sc-97846-SH and TRMT1 shRNA (h) Lentiviral Particles: sc-97846-V as alternate gene silencing products.

For independent verification of TRMT1 (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-97846A and sc-97846B.

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

TRMT1 siRNA (h) is recommended for the inhibition of TRMT1 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

TRMT1 (G-3): sc-373687 is recommended as a control antibody for monitoring of TRMT1 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 TRMT1 gene expression knockdown using RT-PCR Primer: TRMT1 (h)-PR: sc-97846-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.