

TRM61 siRNA (m): sc-154682

BACKGROUND

TRM61 (tRNA m¹A58 methyltransferase subunit TRM61), also known as GCD14, is one of two subunits (the other being TRM6) that function as heterotetramers to comprise the tRNA m¹A58 methyltransferase. The tRNA m¹A58 methyltransferase plays a role in tRNA modification and is specifically responsible for the formation of 1-methyladenosine. 1-methyladenosine is a modified nucleoside found at position 58 in tRNA and is required for maintaining the stability of initiator methionine tRNA (tRNA_i^{Met}) which is directly involved in the initiation of protein synthesis. This implies that TRM61 is crucial for proper tRNA structure and function. Mutations in the gene encoding TRM61 which cause structural changes in the substrate-binding pocket of tRNA m¹A58 methyltransferase can lead to instability of tRNA_i^{Met}.

REFERENCES

1. Anderson, J., Phan, L., Cuesta, R., Carlson, B.A., Pak, M., Asano, K., Björk, G.R., Tamame, M. and Hinnebusch, A.G. 1998. The essential Gcd10p-Gcd14p nuclear complex is required for 1-methyladenosine modification and maturation of initiator methionyl-tRNA. *Genes Dev.* 12: 3650-3662.
2. Calvo, O., Cuesta, R., Anderson, J., Gutierrez, N., García-Barrio, M.T., Hinnebusch, A.G. and Tamame, M. 1999. GCD14p, a repressor of GCN4 translation, cooperates with Gcd10p and Lhp1p in the maturation of initiator methionyl-tRNA in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 19: 4167-4181.
3. Anderson, J., Phan, L. and Hinnebusch, A.G. 2000. The Gcd10p/Gcd14p complex is the essential two-subunit tRNA(1-methyladenosine) methyltransferase of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 97: 5173-5178.
4. Bujnicki, J.M. 2001. In silico analysis of the tRNA: m¹A58 methyltransferase family: homology-based fold prediction and identification of new members from eubacteria and archaea. *FEBS Lett.* 507: 123-127.
5. Kadaba, S., Krueger, A., Trice, T., Krecic, A.M., Hinnebusch, A.G. and Anderson, J. 2004. Nuclear surveillance and degradation of hypomodified initiator tRNA^{Met} in *S. cerevisiae*. *Genes Dev.* 18: 1227-1240.
6. Arhin, G.K., Shen, S., Irmer, H., Ullu, E. and Tschudi, C. 2004. Role of a 300 kDa nuclear complex in the maturation of *Trypanosoma brucei* initiator methionyl-tRNA. *Eukaryot. Cell* 3: 893-899.
7. Ozanick, S., Krecic, A., Andersland, J. and Anderson, J.T. 2005. The bipartite structure of the tRNA m¹A58 methyltransferase from *S. cerevisiae* is conserved in humans. *RNA* 11: 1281-1290.
8. Hiley, S.L., Jackman, J., Babak, T., Trochesset, M., Morris, Q.D., Phizicky, E. and Hughes, T.R. 2005. Detection and discovery of RNA modifications using microarrays. *Nucleic Acids Res.* 33: e2.
9. Ozanick, S.G., Bujnicki, J.M., Sem, D.S. and Anderson, J.T. 2007. Conserved amino acids in each subunit of the heterologous tRNA m¹A58 Mtase from *Saccharomyces cerevisiae* contribute to tRNA binding. *Nucleic Acids Res.* 35: 6808-6819.

CHROMOSOMAL LOCATION

Genetic locus: Trmt61a (mouse) mapping to 12 F1.

PRODUCT

TRM61 siRNA (m) is a target-specific 19-25 nt siRNA 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 TRM61 shRNA Plasmid (m): sc-154682-SH and TRM61 shRNA (m) Lentiviral Particles: sc-154682-V as alternate gene silencing 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

TRM61 siRNA (m) is recommended for the inhibition of TRM61 expression in mouse 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor TRM61 gene expression knockdown using RT-PCR Primer: TRM61 (m)-PR: sc-154682-PR (20 μ l). 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.