

RNMT siRNA (h): sc-75230

BACKGROUND

RNMT (RNA (guanine-7-) methyltransferase), also known as MET, RG7MT1 or hCMT1c, is a widely expressed nuclear protein that belongs to the mRNA cap methyltransferase family. It is responsible for catalyzing the final step in the attachment of the m⁷GpppN cap to the 5' end of mRNA. Capping of mRNA plays an important role in mRNA processing, stability and translation and is therefore important for efficient gene expression. There are three enzymatic steps in the generation of the mRNA cap. The first two steps are catalyzed by RNGTT (RNA guanylyltransferase and 5' phosphatase), and the third step is catalyzed by RNMT. More specifically, RNMT catalyzes the transfer of a methyl group from AdoMet (S-adenosylmethionine) to the GpppN end of the growing mRNA at the N-7 position, thereby producing AdoHyc (S-adenosylhomocysteine) and m⁷GpppN terminated RNA. Two additional isoforms of RNMT exist due to alternative splicing events, namely hCMT1a and hCMT1b.

REFERENCES

1. Pillutla, R.C., et al. 1998. Human mRNA capping enzyme (RNGTT) and cap methyltransferase (RNMT) map to 6q16 and 18p11.22-p11.23, respectively. *Genomics* 54: 351-353.
2. Pillutla, R.C., et al. 1998. Recombinant human mRNA cap methyltransferase binds capping enzyme/RNA polymerase I complexes. *J. Biol. Chem.* 273: 21443-21446.
3. Yokoska, J., et al. 2000. Cloning and characterization of mRNA capping enzyme and mRNA (Guanine-7-)-methyltransferase cDNAs from *Xenopus laevis*. *Biochem. Biophys. Res. Commun.* 268: 617-624.
4. Li, J., et al. 2005. Amino acid residues within conserved domain VI of the vesicular stomatitis virus large polymerase protein essential for mRNA cap methyltransferase activity. *J. Virol.* 79: 13373-13384.
5. Chrebet, G.L., et al. 2005. Cell-based assays to detect inhibitors of fungal mRNA capping enzymes and characterization of sinefungin as a cap methyltransferase inhibitor. *J. Biomol. Screen.* 10: 355-364.
6. Schwer, B., et al. 2006. Genetic analysis of poxvirus mRNA cap methyltransferase: suppression of conditional mutations in the stimulatory D12 subunit by second-site mutations in the catalytic D1 subunit. *Virology* 352: 145-156.

CHROMOSOMAL LOCATION

Genetic locus: RNMT (human) mapping to 18p11.21.

PRODUCT

RNMT 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 RNMT shRNA Plasmid (h): sc-75230-SH and RNMT shRNA (h) Lentiviral Particles: sc-75230-V as alternate gene silencing products.

For independent verification of RNMT (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-75230A, sc-75230B and sc-75230C.

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

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

RNMT (3H3-1D12): sc-517112 is recommended as a control antibody for monitoring of RNMT 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 RNMT gene expression knockdown using RT-PCR Primer: RNMT (h)-PR: sc-75230-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.