SANTA CRUZ BIOTECHNOLOGY, INC.

cSHMT siRNA (m): sc-40941

BACKGROUND

Mammalian serine hydroxymethyltransferase (SHMT) is a tetrameric, pyridoxal phosphate (PLP)-dependent enzyme that catalyzes the reversible interconversion of serine and tetrahydrofolate to glycine and methylenetetrahydrofolate in the cytoplasm (cSHMT, SHMT1) and mitochondria (mSHMT, SHMT2). cSHMT preferentially supplies one-carbon units for thymidylate biosynthesis, depletes methylenetetrahydrofolate pools for S-adenosylmethionine (SAM) synthesis by synthesizing serine, sequesters 5-methyltetrahydrofolate and inhibits SAM synthesis. Sheep liver cytosolic recombinant SHMT (scSHMT) Lys-71, Arg-80 and Asp 89 residues influence intra-subunit ionic interactions essential for catalytic activity; Tyr 72, Asp 227 and His-356 residues in the active site interact with PLP and maintain the tetrameric structure. Human cSHMT and mSHMT genes map to 17p11.2 and 12q13, respectively. The cDNA for the mitochondrial enzyme encodes a mature protein of 474 residues.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM™. 1998. Johns Hopkins University, Baltimore, MD. MIM Number: 138450. World Wide Web URL: http://www.ncbi.nlm. nih.gov/omim
2. Liu, X., et al. 2001. Lack of catalytic activity of a murine mRNA cytoplasmic serine hydroxymethyltransferase splice variant: evidence against alternative splicing as a regulatory mechanism. Biochemistry 40: 4932-4939.
3. Trivedi, V., et al. 2002. Crystal structure of binary and ternary complexes of serine hydroxymethyltransferase from *Bacillus stearothermophilus:* insights into the catalytic mechanism. J. Biol. Chem. 277: 17161-17169.
4. Herbig, K., et al. 2002. Cytoplasmic serine hydroxymethyltransferase mediates competition between folate-dependent deoxyribonucleotide and S-adenosylmethionine biosyntheses. J. Biol. Chem. 277: 38381-38389.
5. Jala, V.R., et al. 2003. Identification of amino acid residues, essential for maintaining the tetrameric structure of sheep liver cytosolic serine hydroxymethyltransferase, by targeted mutagenesis. Biochem. J. 369: 469-476.
6. LocusLink Report (LocusID: 6472). http://www.ncbi.nlm.nih.gov/LocusLink/

CHROMOSOMAL LOCATION

Genetic locus: Shmt1 (mouse) mapping to 11 B2.

PRODUCT

cSHMT siRNA (m) 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 cSHMT shRNA Plasmid (m): sc-40941-SH and cSHMT shRNA (m) Lentiviral Particles: sc-40941-V as alternate gene silencing products.

For independent verification of cSHMT (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-40941A, sc-40941B and sc-40941C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

cSHMT siRNA (m) is recommended for the inhibition of cSHMT 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 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

cSHMT (A-2): sc-365203 is recommended as a control antibody for monitoring of cSHMT 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 cSHMT gene expression knockdown using RT-PCR Primer: cSHMT (m)-PR: sc-40941-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.