

# GNMT siRNA (h): sc-62391

## BACKGROUND

Glycine N-methyltransferase (GNMT) is a 295 amino acid protein that catalyzes the methylation of glycine by using S-adenosylmethionine (AdoMet) to form N-methylglycine (sarcosine) with the concomitant production of S-adenosylhomocysteine (AdoHcy). This process indicates that GNMT probably plays a crucial role in the regulation of tissue concentration of AdoMet and in the metabolism of methionine. Originally identified as a methyl donor, AdoMet is now considered a key metabolite that regulates hepatocyte growth, death and differentiation. Biosynthesis of AdoMet occurs in all mammalian cells as the first step in methionine catabolism in a reaction catalyzed by methionine adenosyltransferase (MAT). Decreased hepatic AdoMet biosynthesis is a consequence of all forms of chronic liver injury. In chronic liver AdoMet deficiency, the liver is predisposed to further injury and can develop spontaneous steatohepatitis and hepatocellular carcinoma. However, impaired AdoMet metabolism, which occurs in patients with mutations of GNMT, can also lead to liver injury.

## REFERENCES

1. Pakhomova, S., et al. 2004. Glycine N-methyltransferases: a comparison of the crystal structures and kinetic properties of recombinant human, mouse and rat enzymes. *Proteins* 57: 331-337.
2. Beagle, B., et al. 2005. The glycine N-methyltransferase (GNMT) 1289 C→T variant influences plasma total homocysteine concentrations in young women after restricting folate intake. *J. Nutr.* 135: 2780-2785.
3. Uthus, E.O., et al. 2006. Differential effects of dietary selenium (se) and folate on methyl metabolism in liver and colon of rats. *Biol. Trace Elem. Res.* 109: 201-214.
4. Velichkova, P., et al. 2006. Methyl transfer in glycine N-methyltransferase. A theoretical study. *J. Phys. Chem. B* 109: 8216-8219.
5. Luka, Z., et al. 2006. A glycine N-methyltransferase knockout mouse model for humans with deficiency of this enzyme. *Transgenic Res.* 15: 393-397.

## CHROMOSOMAL LOCATION

Genetic locus: GNMT (human) mapping to 6p21.1.

## PRODUCT

GNMT 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GNMT shRNA Plasmid (h): sc-62391-SH and GNMT shRNA (h) Lentiviral Particles: sc-62391-V as alternate gene silencing products.

For independent verification of GNMT (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-62391A, sc-62391B and sc-62391C.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

GNMT siRNA (h) is recommended for the inhibition of GNMT 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  $\mu$ M in 66  $\mu$ 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

GNMT (A-4): sc-166834 is recommended as a control antibody for monitoring of GNMT 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 GNMT gene expression knockdown using RT-PCR Primer: GNMT (h)-PR: sc-62391-PR (20  $\mu$ 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.