SANTA CRUZ BIOTECHNOLOGY, INC.

BHMT siRNA (h): sc-91965

BACKGROUND

Betaine-homocysteine methyltransferase (BHMT) is a zinc-dependent cytosolic protein that catalyzes the conversion of betaine and homocysteine to dimethylglycine and methionine, respectively. BHMT is highly expressed in rat liver, and its expression is regulated by dietary methionine and choline. In humans, S-adenosylmethionine (SAM) down-regulates BHMT expression by inducing NFκB, which acts as a repressor for the BHMT gene. Lowered BHMT levels can lead to ER (endoplasmic reticulum) stress. Mutations in the gene encoding for BHMT may lead to hyperhomocysteinemia, a medical condition characterized by abnormally large amounts of homocysteine in the blood which may be a risk factor for cardiovascular and cerebrovascular diseases.

REFERENCES

1. Park, E.I., et al. 1999. Interaction between dietary methionine and methyl donor intake on rat liver betaine-homocysteine methyltransferase gene expression and organization of the human gene. J. Biol. Chem. 274: 7816-7824.
2. Garrow, T.A. 2002. Random mutagenesis of the zinc-binding motif of betaine-homocysteine methyltransferase reveals that Gly 214 is essential. Arch. Biochem. Biophys. 399: 73-80.
3. Evans, J.C., et al. 2002. Betaine-homocysteine methyltransferase: zinc in a distorted barrel. Structure 10: 1159-1171.
4. Forestier, M., et al. 2003. Betaine homocysteine methyltransferase: gene cloning and expression analysis in rat liver cirrhosis. Biochim. Biophys. Acta 1638: 29-34.
5. Weisberg, I.S., et al. 2003. Investigations of a common genetic variant in betaine-homocysteine methyltransferase (BHMT) in coronary artery disease. Atherosclerosis 167: 205-214.
6. Lee, M.B., et al. 2004. A nuclear-magnetic-resonance-based assay for betaine-homocysteine methyltransferase activity. Anal. Biochem. 330: 199-205.
7. Castro, C., et al. 2004. Dissecting the catalytic mechanism of betaine-homocysteine S-methyltransferase by use of intrinsic tryptophan fluorescence and site-directed mutagenesis. Biochemistry 43: 5341-5351.

CHROMOSOMAL LOCATION

Genetic locus: BHMT (human) mapping to 5q14.1.

PRODUCT

BHMT 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see BHMT shRNA Plasmid (h): sc-91965-SH and BHMT shRNA (h) Lentiviral Particles: sc-91965-V as alternate gene silencing products.

For independent verification of BHMT (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-91965A, sc-91965B and sc-91965C.

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

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

BHMT (H-7): sc-390299 is recommended as a control antibody for monitoring of BHMT 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 BHMT gene expression knockdown using RT-PCR Primer: BHMT (h)-PR: sc-91965-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.