SANTA CRUZ BIOTECHNOLOGY, INC.

MTHFS siRNA (m): sc-62650

BACKGROUND

MTHFS (5-formyltetrahydrofolate cyclo-ligase) is a cytosolic protein involved in the formate metabolic process. MTHFS can be account for up to thirty percent of a cells cytoplasmic folate derivitives. MTHFS, with a magnesium cofactor, catalyzes the ATP-dependent reaction that reduces 5-formyltetrahydrofolate to 5,10-methenyltetrahydrofolate. Folate is a necessary molecule for DNA replication and a deficiency in folate can lead to numerous disease states. During DNA replication 5,10-methenyltetrahydrofolate is oxidized and MTHFS is responsible for resetting the molecule.

REFERENCES

1. Anguera, M.C., Liu, X. and Stover, P.J. 2004. Cloning, expression, and purification of 5,10-methenyltetrahydrofolate synthetase from *Mus musculus*. Protein Expr. Purif. 35: 276-283.
2. Chen, S., Yakunin, A.F., Proudfoot, M., Kim, R. and Kim, S.H. 2005. Structural and functional characterization of a 5,10-methenyltetrahydrofolate synthetase from *Mycoplasma pneumoniae* (GI: 13508087). Proteins 61: 433-443.
3. Anguera, M.C. and Stover, P.J. 2006. Methenyltetrahydrofolate synthetase is a high-affinity catecholamine-binding protein. Arch. Biochem. Biophys. 455: 175-187.
4. Field, M.S., Szebenyi, D.M. and Stover, P.J. 2006. Regulation of *de novo* purine biosynthesis by methenyltetrahydrofolate synthetase in neuroblastoma. J. Biol. Chem. 281: 4215-4221.
5. Lee, K.M., Lan, Q., Kricker, A., Purdue, M.P., Grulich, A.E., Vajdic, C.M., Turner, J., Whitby, D., Kang, D., Chanock, S., Rothman, N. and Armstrong, B.K. 2007. One-carbon metabolism gene polymorphisms and risk of non-Hodgkin lymphoma in Australia. Hum. Genet. 122: 525-533.
6. Matakidou, A., El Galta, R., Rudd, M.F., Webb, E.L., Bridle, H., Eisen, T. and Houlston, R.S. 2007. Prognostic significance of folate metabolism polymorphisms for lung cancer. Br. J. Cancer 97: 247-252.

CHROMOSOMAL LOCATION

Genetic locus: Mthfs (mouse) mapping to 9 E3.1.

PRODUCT

MTHFS 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 MTHFS shRNA Plasmid (m): sc-62650-SH and MTHFS shRNA (m) Lentiviral Particles: sc-62650-V as alternate gene silencing products.

For independent verification of MTHFS (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-62650A, sc-62650B and sc-62650C.

PROTOCOLS

See our web site at 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 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

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

MTHFS (G-8): sc-398056 is recommended as a control antibody for monitoring of MTHFS 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 MTHFS gene expression knockdown using RT-PCR Primer: MTHFS (m)-PR: sc-62650-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.