SANTA CRUZ BIOTECHNOLOGY, INC.

MTHFD1L siRNA (m): sc-149679

BACKGROUND

Methylenetetrahydrofolate dehydrogenase 1 (MTHFD1) is a 935-amino acid, folate-dependent protein that is responsible for the consecutive interconversion of tetrahydrofolate derivatives which drive the synthesis of purine, methionine and thymidylate. MTHFD1 functions as a homodimer consisting of two major domains; an N-terminal domain containing the dehydrogenase and cyclohydrolase activities and a larger synthetase domain in the C-terminus. Mutations in the MTHFD1 gene in pregnant women are associated with an increased risk of giving birth to a child with a neural tube defect, along with a possible risk of decreased embryo survival. MTHFD1L (methylenetetrahydrofolate dehydrogenase 1-like) is a 978 amino acid mitochondrial protein that is expressed in a variety of tissues and, like MTHFD1, functions in folate metabolism via the tetrahydrofolate pathway. MTHFD1L exists as two isoforms and may be associated with colorectal carcinogenesis, possibly conferring a growth advantage to cancer-transformed cells.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 611427. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
2. Prasannan, P., et al. 2003. Human mitochondrial C1-tetrahydrofolate synthase: gene structure, tissue distribution of the mRNA, and immunolocalization in Chinese hamster ovary calls. J. Biol. Chem. 278: 43178-43187.
3. Sugiura, T., et al. 2004. A novel mitochondrial C1-tetrahydrofolate synthetase is upregulated in human colon adenocarcinoma. Biochem. Biophys. Res. Commun. 315: 204-211.
4. Krajinovic, M., et al. 2004. Role of polymorphisms in MTHFR and MTHFD1 genes in the outcome of childhood acute lymphoblastic leukemia. Pharmacogenomics J. 4: 66-72.
5. Walkup, A.S., et al. 2005. Enzymatic characterization of human mitochondrial C1-tetrahydrofolate synthase. Arch. Biochem. Biophys. 442: 196-205.
6. Christensen, K.E., et al. 2005. Disruption of the MTHFD1 gene reveals a monofunctional 10-formyltetrahydrofolate synthetase in mammalian mitochondria. J. Biol. Chem. 280: 7597-7602.

CHROMOSOMAL LOCATION

Genetic locus: Mthfd1l (mouse) mapping to 10 A1.

PRODUCT

MTHFD1L 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 MTHFD1L shRNA Plasmid (m): sc-149679-SH and MTHFD1L shRNA (m) Lentiviral Particles: sc-149679-V as alternate gene silencing products.

For independent verification of MTHFD1L (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-149679A, sc-149679B and sc-149679C.

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

MTHFD1L siRNA (m) is recommended for the inhibition of MTHFD1L 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

MTHFD1L (6C7): sc-100783 is recommended as a control antibody for monitoring of MTHFD1L 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 MTHFD1L gene expression knockdown using RT-PCR Primer: MTHFD1L (m)-PR: sc-149679-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.