SANTA CRUZ BIOTECHNOLOGY, INC.

MTHFD1 siRNA (h): sc-61082

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. The cytosolic MRHFD1 contains three subunits, 5,10-methylenetetrahydrofolate dehydrogenase, 5,10-methenyltetrahydrofolate cyclohydrolase, and 10-formyltetrahydrofolate synthetase, each with distinct activities. MTHFD1 functions as a homodimer consisting of two major domains, an N-terminal 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. MTHFD1 also plays a role in migraine development, since folate metabolism is involved in migraine pathophysiology, mainly in migraine with aura.

REFERENCES

1. Arakawa, T. 1970. Congenital defects in folate utilization. Am. J. Med. 48: 594-598.
2. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 172460. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
3. 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.
4. 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.
5. Mills, J.L., et al. 2005. Folate-related genes and omphalocele. Am. J. Med. Genet. A 136: 8-11.
6. Oterino, A., et al. 2005. Thymidylate synthase promoter tandem repeat and MTHFD1 R653Q polymorphisms modulate the risk for migraine conferred by the MTHFR T677 allele. Brain Res. Mol. Brain Res. 139: 163-168.

CHROMOSOMAL LOCATION

Genetic locus: MTHFD1 (human) mapping to 14q23.3.

PRODUCT

MTHFD1 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 MTHFD1 shRNA Plasmid (h): sc-61082-SH and MTHFD1 shRNA (h) Lentiviral Particles: sc-61082-V as alternate gene silencing products.

For independent verification of MTHFD1 (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-61082A, sc-61082B and sc-61082C.

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

MTHFD1 siRNA (h) is recommended for the inhibition of MTHFD1 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

MTHFD1 (A-8): sc-271412 is recommended as a control antibody for monitoring of MTHFD1 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 MTHFD1 gene expression knockdown using RT-PCR Primer: MTHFD1 (h)-PR: sc-61082-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Shao, C., et al. 2020. Cytosolic ME1 integrated with mitochondrial IDH2 supports tumor growth and metastasis. Redox Biol. 36: 101685.

RESEARCH USE

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