

MUT siRNA (m): sc-149723

BACKGROUND

MUT (methylmalonyl coenzyme A mutase), also known as MCM, is a 750 amino acid mitochondrial matrix protein that exists as a homodimer and belongs to the methylmalonyl-CoA mutase family. Induced by adenosylcobalamin (also known as coenzyme B₁₂ or vitamin B₁₂), MUT participates in the degradation of various amino acids, odd-chain fatty acids and cholesterol via propionyl-CoA (PCC) during the tricarboxylic acid cycle. Mutations in the gene encoding MUT, which is located on human chromosome 6, are the cause of methylmalonic aciduria type mut (MMAM), an often fatal disorder of organic acid metabolism that is characterized by lethargy, vomiting, failure to thrive, hypotonia, neurological deficit and early death. Two forms of MMAM exists: mut(o), which there is no detectable enzymatic activity and mut(-), which there is residual cobalamin-dependent activity.

REFERENCES

1. Wilkemeyer, M.F., et al. 1991. Differential diagnosis of mut and cbl methylmalonic aciduria by DNA-mediated gene transfer in primary fibroblasts. *J. Clin. Invest.* 87: 915-918.
2. Crane, A.M., et al. 1992. Cloning and expression of a mutant methylmalonyl coenzyme A mutase with altered cobalamin affinity that causes mut- methylmalonic aciduria. *J. Clin. Invest.* 89: 385-391.
3. Crane, A.M. and Ledley, F.D. 1994. Clustering of mutations in methylmalonyl CoA mutase associated with mut- methylmalonic acidemia. *Am. J. Hum. Genet.* 55: 42-50.
4. Treacy, E., et al. 1996. Glutathione deficiency as a complication of methylmalonic acidemia: response to high doses of ascorbate. *J. Pediatr.* 129: 445-448.
5. Janata, J., et al. 1997. Expression and kinetic characterization of methylmalonyl-CoA mutase from patients with the mut- phenotype: evidence for naturally occurring interallelic complementation. *Hum. Mol. Genet.* 6: 1457-1464.
6. Ledley, F.D. and Rosenblatt, D.S. 1997. Mutations in mut methylmalonic acidemia: clinical and enzymatic correlations. *Hum. Mutat.* 9: 1-6.

CHROMOSOMAL LOCATION

Genetic locus: Mut (mouse) mapping to 17 B2.

PRODUCT

MUT 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see MUT shRNA Plasmid (m): sc-149723-SH and MUT shRNA (m) Lentiviral Particles: sc-149723-V as alternate gene silencing products.

For independent verification of MUT (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-149723A, sc-149723B and sc-149723C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

MUT siRNA (m) is recommended for the inhibition of MUT 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 μ M in 66 μ 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

MUT (D-1): sc-390978 is recommended as a control antibody for monitoring of MUT 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 κ BP-HRP: sc-516102 or m-IgG κ 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 κ BP-FITC: sc-516140 or m-IgG κ 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 MUT gene expression knockdown using RT-PCR Primer: MUT (m)-PR: sc-149723-PR (20 μ 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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.