

MMAB siRNA (m): sc-75803

BACKGROUND

MMAB (methylmalonic aciduria (cobalamin deficiency) type B protein), also known as ATR or Cob(I)alamin adenosyltransferase, is a mitochondrial protein expressed in skeletal muscle and liver. MMAB belongs to the Cob(I)alamin adenosyltransferase family and plays an important role in adenosylcobalamin (AdoCbl) biosynthesis. More specifically, MMAB catalyzes the final step in the biosynthesis pathway: the conversion of vitamin B12 (also known as cobalamin) to AdoCbl. AdoCbl is an essential cofactor utilized by MUT, the mitochondrial methylmalonyl-CoA mutase that plays an important role in the catabolism of cholesterol, branched chain amino acids, odd-numbered fatty acids and other metabolites. Mutations in the gene encoding MMAB can result in methylmalonic aciduria type B (MMAB), also known as vitamin B12-responsive methylmalonic aciduria of cblB complementation type. The autosomal recessive MMAB disease is characterized by defective synthesis of AdoCbl.

REFERENCES

1. Johnson, C.L., et al. 2001. Functional genomic, biochemical, and genetic characterization of the *Salmonella* pduO gene, an ATP:cob(I)alamin adenosyltransferase gene. *J. Bacteriol.* 183: 1577-1584.
2. Dobson, C.M., et al. 2002. Identification of the gene responsible for the cblB complementation group of vitamin B12-dependent methylmalonic aciduria. *Hum. Mol. Genet.* 11: 3361-3369.
3. Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 607568. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
4. Saridakis, V., et al. 2004. The structural basis for methylmalonic aciduria. The crystal structure of archaeal ATP:cobalamin adenosyltransferase. *J. Biol. Chem.* 279: 23646-23653.
5. Lerner-Ellis, J.P., et al. 2006. Mutation and biochemical analysis of patients belonging to the cblB complementation class of vitamin B12-dependent methylmalonic aciduria. *Mol. Genet. Metab.* 87: 219-225.

CHROMOSOMAL LOCATION

Genetic locus: Mmab (mouse) mapping to 5 F.

PRODUCT

MMAB 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 MMAB shRNA Plasmid (m): sc-75803-SH and MMAB shRNA (m) Lentiviral Particles: sc-75803-V as alternate gene silencing products.

For independent verification of MMAB (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-75803A, sc-75803B and sc-75803C.

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 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

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

MMAB (G-3): sc-271424 is recommended as a control antibody for monitoring of MMAB 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 MMAB gene expression knockdown using RT-PCR Primer: MMAB (m)-PR: sc-75803-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.