SANTA CRUZ BIOTECHNOLOGY, INC.

MCCB siRNA (m): sc-60999

BACKGROUND

Methylcrotonoly-CoA carboxylase β chain, or MCCB, is the non-biotin containing subunit of the MCC enzyme. The deduced 563 amino acid polypeptide contains an N-terminal mitochondrial targeting sequence. MCCB is a putative dodecamer composed of six biotin-containing α subunits and six β subunits. MCCB plays a role in leucine catabolism and catalyzes the conversion of 3-methylcrotonoyl-CoA to 3-methylglutaconyl-CoA, using ATP as an energy source. Defects in the MCCC2 gene, which encodes MCCB, cause 3-methylcrotonylglycinuria type II (MCGII), a recessive disease characterized by muscular hypotonia and atrophy, probably of spinal origin.

REFERENCES

1. Bannwart, C., Wermuth, B., Baumgartner, R., Suormala, T. and Weismann, U.N. 1993. Isolated biotin-resistant deficiency of 3-methylcrotonyl-CoA carboxylase presenting as a clinically severe form in a newborn with fatal outcome. J. Inherit. Metab. Dis. 15: 863-868.
2. Baumgartner, M.R., Almashanu, S., Suormala, T., Obie, C., Cole, R.N., Packman, S., Baumgartner, E.R. and Valle, D. 2001. The molecular basis of human 3-methylcrotonyl-CoA carboxylase deficiency. J. Clin. Investig. 107: 495-504.
3. Gallardo, M.E., Desviat, L.R., Rodríguez, J.M., Esparza-Gordillo, J., Perez-Cerdá, C., Perez, B., Rodríguez-Pombo, P., Criado, O., Sanz, R., Morton, D.H., Gibson, K.M., Le, T.P., Ribes, A., de Córdoba, S.R., Ugarte, M. and Peñalva, M.A. 2001. The molecular basis of 3-methylcrotonylglycinuria, a disorder of leucine catabolism. Am. J. Hum. Genet. 68: 334-346.
4. Holzinger, A., Röschinger, W., Lagler, F., Mayerhofer, P.U., Lichtner, P., Kattenfeld, T., Thuy, L.P., Nyhan, W.L., Koch, H.G., Muntau, A.C. and Roscher, A.A. 2001. Cloning of the human MCCA and MCCB genes and mutations therein reveal the molecular cause of 3-methylcrotonyl-CoA: carboxylase deficiency. Hum. Mol. Genet. 10: 1299-1306.

CHROMOSOMAL LOCATION

Genetic locus: Mccc2 (mouse) mapping to 13 D1.

PRODUCT

MCCB 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 MCCB shRNA Plasmid (m): sc-60999-SH and MCCB shRNA (m) Lentiviral Particles: sc-60999-V as alternate gene silencing products.

For independent verification of MCCB (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-60999A, sc-60999B and sc-60999C.

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

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

MCCB (B-1): sc-390836 is recommended as a control antibody for monitoring of MCCB 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 MCCB gene expression knockdown using RT-PCR Primer: MCCB (m)-PR: sc-60999-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.