SANTA CRUZ BIOTECHNOLOGY, INC.

BCKDE1A siRNA (m): sc-77386

BACKGROUND

BCKDE1A (branched-chain α-keto acid dehydrogenase E1 component α chain), also known as BCKDHA or 2-oxoisovalerate dehydrogenase subunit α, is part of the inner mitochondrial membrane complex involved in the catabolism of the branched-chain amino acids. This complex consists of multiple copies of three catalytic components: BCKDE1, DBT and DLD. It is responsible for catalyzing the conversion of α-keto acids to acyl-CoA and CO_2. BCKDE1A is the α chain component of BCKDE1. BCKDE1 is heterotetrameric, consisting of two α chains and two β chains. A mutation in BCKDE1A can result in a deficiency of the properly assembled BCKDH complex. A deficiency of this enzyme leads to an accumulation of branched-chain amino acids in the blood an urine. This metabolic disorder is called type IA maple syrup urine disease (MSUD).

REFERENCES

1. Chuang, J.L., et al. 1994. Molecular basis of maple syrup urine disease: novel mutations at the E1 α locus that impair E1(α_2 β_2) assembly or decrease steady-state E1 α mRNA levels of branched-chain α-keto acid dehydrogenase complex. Am. J. Hum. Genet. 55: 297-304.
2. Simon, E., et al. 2005. Maple syrup urine disease-treatment and outcome in patients of Turkish descent in Germany. Turk. J. Pediatr. 47: 8-13.
3. Hallam, P., et al. 2005. A new protein substitute for adolescents and adults with maple syrup urine disease (MSUD). J. Inherit. Metab. Dis. 28: 665-672.
4. Funchal, C., et al. 2005. Morphological alterations and cell death provoked by the branched-chain α-amino acids accumulating in maple syrup urine disease in astrocytes from rat cerebral cortex. Cell. Mol. Neurobiol. 25: 851-867.
5. Funchal, C., et al. 2005. Evidence that intracellular Ca^{2+} mediates the effect of α-ketoisocaproic acid on the phosphorylating system of cytoskeletal proteins from cerebral cortex of immature rats. J. Neurol. Sci. 238: 75-82.
6. Bridi, R., et al. 2005. α-keto acids accumulating in maple syrup urine disease stimulate lipid peroxidation and reduce antioxidant defences in cerebral cortex from young rats. Metab. Brain Dis. 20: 155-167.
7. Funchal, C., et al. 2005. Branched-chain α-keto acids accumulating in maple syrup urine disease induce reorganization of phosphorylated GFAP in C6-glioma cells. Metab. Brain Dis. 20: 205-217.

CHROMOSOMAL LOCATION

Genetic locus: Bckdha (mouse) mapping to 7 A3.

PRODUCT

BCKDE1A 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 BCKDE1A shRNA Plasmid (m): sc-77386-SH and BCKDE1A shRNA (m) Lentiviral Particles: sc-77386-V as alternate gene silencing products.

For independent verification of BCKDE1A (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-77386A, sc-77386B and sc-77386C.

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

BCKDE1A siRNA (m) is recommended for the inhibition of BCKDE1A 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

BCKDE1A (H-5): sc-271538 is recommended as a control antibody for monitoring of BCKDE1A 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 BCKDE1A gene expression knockdown using RT-PCR Primer: BCKDE1A (m)-PR: sc-77386-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.