SANTA CRUZ BIOTECHNOLOGY, INC.

BCKDK siRNA (h): sc-93313

BACKGROUND

BCKDK (branched chain ketoacid dehydrogenase kinase), also known as BCKDHKIN, is a 412 amino acid mitochondrial matrix protein that exists as a monomer and contains one histidine kinase domain. Expressed ubiquitously, BCKDK catalyzes the ATP-dependent phosphorylation and subsequent inactivation of the branched-chain α-ketoacid dehydrogenase (BCKD) complex, a regulatory enzyme complex that plays a crucial role in the catabolic pathways of valine, leucine and isoleucine. Specifically, the BCKD complex functions as the second enzyme in branched-chain amino acid (BCAA) catabolism, effectively catalyzing the irreversible oxidative decarboxylation of BCAAs. Due to the ability of BCKDK to regulate the activity of the BCKD complex, BCKDK plays an essential role in the catabolic pathways of branched-chain amino acid metabolism.

REFERENCES

1. Popov, K.M., et al. 1992. Branched-chain α-ketoacid dehydrogenase kinase. Molecular cloning, expression, and sequence similarity with histidine protein kinases. J. Biol. Chem. 267: 13127-13130.
2. Popov, K.M., et al. 1997. Mitochondrial α-ketoacid dehydrogenase kinases: a new family of protein kinases. Adv. Second Messenger Phosphoprotein Res. 31: 105-111.
3. Suryawan, A., et al. 1998. A molecular model of human branched-chain amino acid metabolism. Am. J. Clin. Nutr. 68: 72-81.
4. Machius, M., et al. 2001. Structure of rat BCKD kinase: nucleotide-induced domain communication in a mitochondrial protein kinase. Proc. Natl. Acad. Sci. USA 98: 11218-11223.
5. Chang, C.F., et al. 2002. Solution structure and dynamics of the lipoic acid-bearing domain of human mitochondrial branched-chain α-keto acid dehydrogenase complex. J. Biol. Chem. 277: 15865-15873.

CHROMOSOMAL LOCATION

Genetic locus: BCKDK (human) mapping to 16p11.2.

PRODUCT

BCKDK 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 BCKDK shRNA Plasmid (h): sc-93313-SH and BCKDK shRNA (h) Lentiviral Particles: sc-93313-V as alternate gene silencing products.

For independent verification of BCKDK (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-93313A, sc-93313B and sc-93313C.

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

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

BCKDK (F-10): sc-374424 is recommended as a control antibody for monitoring of BCKDK 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 BCKDK gene expression knockdown using RT-PCR Primer: BCKDK (h)-PR: sc-93313-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.