SANTA CRUZ BIOTECHNOLOGY, INC.

CARKL siRNA (m): sc-142012

BACKGROUND

CARKL, also known as SHPK (Sedoheptulokinase), is a 478 amino acid protein that localizes to the cytoplasm and belongs to the FGGY family of protein kinases. Expressed at high levels in kidney, pancreas and liver and at lower levels in heart, placenta, brain and lung, CARKL functions at an optimal pH of 8.5 and catalyzes the ATP-dependent phosphorylation of sedoheptulose to yield sedoheptulose 7-phosphate, an intermediate in the pentose phosphate pathway. Once phosphorylated, sedoheptulose is unable to exit the cell via the cell membrane, resulting in the containment of sedoheptulose 7-phosphate within the cell. Defects in the gene encoding CARKL are associated with cystinosis, an autosomal recessive genetic disorder of the renal tubules that is characterized by excessive urination and low blood levels of phosphates and potassium.

REFERENCES

1. Williams, J.F., et al. 1985. The significance of sedoheptulose 1,7-bisphosphate in the metabolism and regulation of the pentose pathway in liver. Biochem. Int. 11: 599-610.
2. Anikster, Y., et al. 1999. CTNS mutations in patients with cystinosis. Hum. Mutat. 14: 454-458.
3. Touchman, J.W., et al. 2000. The genomic region encompassing the nephropathic cystinosis gene (CTNS): complete sequencing of a 200-kb segment and discovery of a novel gene within the common cystinosis-causing deletion. Genome Res. 10: 165-173.
4. Phornphutkul, C., et al. 2001. The promoter of a lysosomal membrane transporter gene, CTNS, binds Sp-1, shares sequences with the promoter of an adjacent gene, CARKL, and causes cystinosis if mutated in a critical region. Am. J. Hum. Genet. 69: 712-721.
5. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 605060. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

CHROMOSOMAL LOCATION

Genetic locus: Shpk (mouse) mapping to 11 B4.

PRODUCT

CARKL 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 CARKL shRNA Plasmid (m): sc-142012-SH and CARKL shRNA (m) Lentiviral Particles: sc-142012-V as alternate gene silencing products.

For independent verification of CARKL (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-142012A, sc-142012B and sc-142012C.

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

CARKL siRNA (m) is recommended for the inhibition of CARKL 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CARKL gene expression knockdown using RT-PCR Primer: CARKL (m)-PR: sc-142012-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.