SANTA CRUZ BIOTECHNOLOGY, INC.

PKLR siRNA (m): sc-62819

BACKGROUND

In mammals, four different isoenzymes exist for pyruvate kinase. Based on their tissue distribution, the isoenzymes are designated L-type (for predominant expression in the liver), R-type (for predominant expression in red blood cells), M1-type (for predominant expression in muscle, brain and heart) and M2-type (for predominant expression in fetal tissues). Pyruvate kinases are responsible for catalyzing the final step in glycolysis: the conversion of phosphoenolpyruvate to pyruvate with the coinciding generation of ATP. The PKLR (pyruvate kinase, liver and RBC) gene encodes the L- and R-type isoenzymes through alternative splicing events under the control of different promoters. The R-type isoform, also known as RPK (R-type pyruvate kinase), exists as a tetramer and, when functioning improperly, can result in chronic/hereditary nonspherocytic hemolytic anemia (CNSHA/HNSHA) or pyruvate kinase hyperactivity (also called high red cell ATP syndrome). The L-type isoform, alternatively known as PKL (pyruvate kinase L-type), also exists as a tetramer and is upregulated by glucose with implications in maturity-onset diabetes of the young (MODY).

REFERENCES

1. Tani, K., et al. 1987. Human liver type pyruvate kinase: cDNA cloning and chromosomal assignment. Biochem. Biophys. Res. Commun. 143: 431-438.
2. Tani, K., et al. 1988. Two homozygous cases of erythrocyte pyruvate kinase (PK) deficiency in Japan: PK Sendai and PK Shinshu. Am. J. Hematol. 28: 186-190.
3. Nordström, L. and Lerner, S.A. 1991. Single daily dose therapy with aminoglycosides. J. Hosp. Infect. Suppl. 18A: 117-129.
4. Wang, H., et al. 2002. Liver pyruvate kinase polymorphisms are associated with type 2 diabetes in northern European Caucasians. Diabetes 51: 2861-2865.
5. van Wijk, R., et al. 2003. Disruption of a novel regulatory element in the erythroid-specific promoter of the human PKLR gene causes severe pyruvate kinase deficiency. Blood 101: 1596-1602.
6. Park-Hah, J.O., et al. 2005. A novel homozygous mutation of PKLR gene in a pyruvate-kinase-deficient Korean family. Acta Haematol. 113: 208-211.

CHROMOSOMAL LOCATION

Genetic locus: Pklr (mouse) mapping to 3 F1.

PRODUCT

PKLR 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 PKLR shRNA Plasmid (m): sc-62819-SH and PKLR shRNA (m) Lentiviral Particles: sc-62819-V as alternate gene silencing products.

For independent verification of PKLR (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-62819A, sc-62819B and sc-62819C.

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

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

PKLR (E-2): sc-133222 is recommended as a control antibody for monitoring of PKLR 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 PKLR gene expression knockdown using RT-PCR Primer: PKLR (m)-PR: sc-62819-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.