



PKM siRNA (m): sc-62821

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 PKM (pyruvate kinase, muscle) gene encodes the M1- and M2-type isoenzymes through alternative splicing events. Both M1- and M2-type isoforms exist as tetramers and are stimulated by fructose 1,6-bisphosphate. In addition, both isoforms exhibit thyroid hormone binding activity and may be referred to as CTHBP (cytosolic thyroid hormone-binding protein) or THBP1. The M2-type isoform also interacts with Oct-4 via its C-terminal domain, functioning to enhance Oct-4 transcriptional activity.

REFERENCES

1. Parkison, C., et al. 1991. The monomer of pyruvate kinase, subtype M1, is both a kinase and a cytosolic thyroid hormone binding protein. *Biochem. Biophys. Res. Commun.* 179: 668-674.
2. Ashizawa, K., et al. 1991. *In vivo* regulation of monomer-tetramer conversion of pyruvate kinase subtype M2 by glucose is mediated via fructose 1,6-bisphosphate. *J. Biol. Chem.* 266: 16842-16846.
3. Li, Y., et al. 2005. High glucose upregulates pantothenate kinase 4 (Pank4) and thus affects M2-type pyruvate kinase (Pkm2). *Mol. Cell. Biochem.* 277: 117-125.

CHROMOSOMAL LOCATION

Genetic locus: Pkm2 (mouse) mapping to 9 B.

PRODUCT

PKM 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PKM shRNA Plasmid (m): sc-62821-SH and PKM shRNA (m) Lentiviral Particles: sc-62821-V as alternate gene silencing products.

For independent verification of PKM (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-62821A, sc-62821B and sc-62821C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

PKM siRNA (m) is recommended for the inhibition of PKM 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 μ M in 66 μ l. 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

PKM (C-11): sc-365684 is recommended as a control antibody for monitoring of PKM 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 PKM gene expression knockdown using RT-PCR Primer: PKM (m)-PR: sc-62821-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Wittwer, J.A., et al. 2011. Enhancing mitochondrial respiration suppresses tumor promoter TPA-induced PKM2 expression and cell transformation in skin epidermal JB6 cells. *Cancer Prev. Res.* 4: 1476-1484.
2. Li, R.J.W., et al. 2023. A glucose-sensing mechanism with glucose transporter 1 and pyruvate kinase in the area postrema regulates hepatic glucose production in rats. *J. Biol. Chem.* 299: 104633.

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.