

PFKP siRNA (m): sc-152181

BACKGROUND

Phosphofructokinases (PFKs) are regulatory glycolytic enzymes which catalyze the irreversible conversion of fructose-6-phosphate to fructose-1,6-bisphosphate, an intermediate in the pathway of glycolysis. Mammalian PFK is a tetramer made of three subunits, namely muscle (PFK-1), liver (PFKL) and platelet (PFKP) phosphofructokinase. PFKP (phosphofructokinase, platelet), also known as PFKF or PFK-C, is a 784 amino acid subunit of the PFK complex. Using magnesium as a cofactor, PFKP functions as an allosteric enzyme that, together with other PFK subunits, catalyzes the ATP-dependent phosphorylation of fructose-6-phosphate. Defects in the gene encoding PFKP may lead to an increased risk of obesity, as PFKP plays a crucial role in carbohydrate metabolism.

REFERENCES

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2. Nakajima, H., et al. 1987. Cloning of human muscle phosphofructokinase cDNA. *FEBS Lett.* 223: 113-116.
3. Simpson, C.J. and Fothergill-Gilmore, L.A. 1991. Isolation and sequence of a cDNA encoding human platelet phosphofructokinase. *Biochem. Biophys. Res. Commun.* 180: 197-203.
4. Adam, G.C., et al. 2002. Trifunctional chemical probes for the consolidated detection and identification of enzyme activities from complex proteomes. *Mol. Cell. Proteomics* 1: 828-835.
5. Mahlkecht, U., et al. 2003. Cloning and chromosomal characterization of the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 gene (PFKFB3, iPFK2). *Int. J. Oncol.* 23: 883-891.
6. Hannemann, A., et al. 2005. Characterization of the human P-type 6-phosphofructo-1-kinase gene promoter in neural cell lines. *Gene* 345: 237-247.
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CHROMOSOMAL LOCATION

Genetic locus: Pfkp (mouse) mapping to 13 A1.

PRODUCT

PFKP 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 PFKP shRNA Plasmid (m): sc-152181-SH and PFKP shRNA (m) Lentiviral Particles: sc-152181-V as alternate gene silencing products.

For independent verification of PFKP (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-152181A, sc-152181B and sc-152181C.

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

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

PFKP (F-7): sc-514824 is recommended as a control antibody for monitoring of PFKP 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 PFKP gene expression knockdown using RT-PCR Primer: PFKP (m)-PR: sc-152181-PR (20 μ l). 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.