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PFK-1 shRNA (m) Lentiviral Particles: sc-44562-V

BACKGROUND

Phosphofructokinases (PFK) are regulatory glycolytic enzymes that convert fructose 6-phosphate and ATP into fructose 1,6-bisphosphate (through PFK-1), fructose 2,6-bisphosphate (through PFK-2), and ADP. Human PFK-1 is tetrameric and isoenzymes include, PFK-1 muscle (PFKM, PFK-A), PFK-1 liver (PFKL, PFK-B), and PFK-1 platelet (PFKP, PFK-C, PFKF). PFK-1 is inhibited by ATP and citrate (from the tricarboxylic acid cycle). PFK-1 undergoes activation in the presence of elevated AMP. The most potent activator is fructose-2,6-bisphosphate, which is produced by PFK-2 from the same substrate, fructose 6-phosphate. PFK-2 is bifunctional and a key regulator for PFK-1. PFK-2 catalyzes the synthesis of fructose-2,6-bisphosphate, and contains fructose-2,6-bisphosphatase activity that catalyzes the degradation of fructose-2,6-bisphosphate. PFK-2 is dimeric and isoenzymes include PFK-2 liver (PFKFB1, PFRX), PFK-2 cardiac (PFKFB2), PFK-2 placental (PFKFB3, Inducible PFK-2) and PFK-2 testis (PFKFB4).

REFERENCES

1. Tsuura, Y., et al. 1998. Endogenous nitric oxide inhibits glucose-induced Insulin secretion by suppression of phosphofructokinase activity in pancreatic islets. *Biochem. Biophys. Res. Commun.* 252: 34-38.
2. Chang, S.H., et al. 2002. Role of Ser530, Arg292, and His662 in the allosteric behavior of rabbit muscle phosphofructokinase. *Biochem. Biophys. Res. Commun.* 290: 670-675.
3. Zeitschel, U., et al. 2002. Changes in activity and expression of phosphofructokinase in different rat brain regions after basal forebrain cholinergic lesion. *J. Neurochem.* 83: 371-380.
4. Su, Y., et al. 2003. The α -subunit of the V-type H⁺-ATPase interacts with phosphofructokinase-1 in humans. *J. Biol. Chem.* 278: 20013-20018.

CHROMOSOMAL LOCATION

Genetic locus: Pfk (mouse) mapping to 15 F1.

PRODUCT

PFK-1 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see PFK-1 siRNA (m): sc-44562 and PFK-1 shRNA Plasmid (m): sc-44562-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

APPLICATIONS

PFK-1 shRNA (m) Lentiviral Particles is recommended for the inhibition of PFK-1 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

PFK-1 (E-4): sc-377346 is recommended as a control antibody for monitoring of PFK-1 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PFK-1 gene expression knockdown using RT-PCR Primer: PFK-1 (m)-PR: sc-44562-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.