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SANTA CRUZ BIOTECHNOLOGY, INC.

PFK-2 car siRNA (m): sc-44676

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-biphosphatase 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 Ser 530, Arg 292, and His 662 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.
5. Sotgia, F., et al. 2003. Phosphofructokinase muscle-specific isoform requires caveolin-3 expression for plasma membrane recruitment and caveolar targeting: implications for the pathogenesis of caveolin-related muscle diseases. Am. J. Pathol. 163: 2619-2634.

CHROMOSOMAL LOCATION

Genetic locus: Pfkfb2 (mouse) mapping to 1 E4.

PRODUCT

PFK-2 car 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 PFK-2 car shRNA Plasmid (m): sc-44676-SH and PFK-2 car shRNA (m) Lentiviral Particles: sc-44676-V as alternate gene silencing products.

For independent verification of PFK-2 car (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-44676A, sc-44676B and sc-44676C.

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

PFK-2 car siRNA (m) is recommended for the inhibition of PFK-2 car 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

PFK-2 car (D-1): sc-377416 is recommended as a control antibody for monitoring of PFK-2 car 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 PFK-2 car gene expression knockdown using RT-PCR Primer: PFK-2 car (m)-PR: sc-44676-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.