SANTA CRUZ BIOTECHNOLOGY, INC.

PFKFB2 siRNA (h): sc-44675

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, and 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.
6. Haller, R.G., et al. 2004. No spontaneous second wind in muscle phosphofructokinase deficiency. Neurology 62: 82-86.

CHROMOSOMAL LOCATION

Genetic locus: PFKFB2 (human) mapping to 1q32.2.

PRODUCT

PFKFB2 siRNA (h) 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 PFKFB2 shRNA Plasmid (h): sc-44675-SH and PFKFB2 shRNA (h) Lentiviral Particles: sc-44675-V as alternate gene silencing products.

For independent verification of PFKFB2 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44675A, sc-44675B and sc-44675C.

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

PFKFB2 siRNA (h) is recommended for the inhibition of PFKFB2 expression in human 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

PFKFB2 (D-1): sc-377416 is recommended as a control antibody for monitoring of PFKFB2 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 PFKFB2 gene expression knockdown using RT-PCR Primer: PFKFB2 (h)-PR: sc-44675-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.