SANTA CRUZ BIOTECHNOLOGY, INC.

PFKFB4 siRNA (h): sc-78392

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. PFK-2 tes (6PF-2-K/Fru-2,6-P2ASE testis-type isozyme), also known as PFKFB4 (6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4) is a 469 amino acid cytoplasmic enzyme that is involved in the degradation and synthesis of fructose 2,6-bisphosphate. Specifically expressed in testis, PFKFB4 functions as a homodimer and is regulated via phosphorylation. Expression of PFKFB4 is upregulated in response to hypoxic conditions in a HIF-1α dependent mechanism. Significantly, expression of PFKFB4 is observed in a variety of cancer cell lines, suggesting that it may play a role in the Warburg effect, the observation that malignant cells produce ATP via glycolysis followed by lactic acid fermentation in the cytosol, rather than via pyruvate in the mitochondria.

REFERENCES

1. Sakai, A., et al. 1996. Cloning of cDNA encoding for a novel isozyme of fructose 6-phosphate, 2-kinase/fructose 2,6-bisphosphatase from human placenta. J. Biochem. 119: 506-511.
2. Manzano, A., et al. 1999. Cloning, expression and chromosomal localization of a human testis 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase gene. Gene 229: 83-89.
3. Online Mendelian Inheritance in Man, OMIM™. 2000. Johns Hopkins University, Baltimore, MD. MIM Number: 605320. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
4. Minchenko, O.H., et al. 2005. Expression and hypoxia-responsiveness of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 in mammary gland malignant cell lines. Acta Biochim. Pol. 52: 881-888.
5. Minchenko, O.H., et al. 2005. Overexpression of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-4 in the human breast and colon malignant tumors. Biochimie 87: 1005-1010.
6. Gómez, M., et al. 2005. Specific expression of pfkfb4 gene in spermatogonia germ cells and analysis of its 5'-flanking region. FEBS Lett. 579: 357-362.

CHROMOSOMAL LOCATION

Genetic locus: PFKFB4 (human) mapping to 3p21.31.

PRODUCT

PFKFB4 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 PFKFB4 shRNA Plasmid (h): sc-78392-SH and PFKFB4 shRNA (h) Lentiviral Particles: sc-78392-V as alternate gene silencing products.

For independent verification of PFKFB4 (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-78392A, sc-78392B and sc-78392C.

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

PFKFB4 siRNA (h) is recommended for the inhibition of PFKFB4 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

PFKFB4 (A-1): sc-514792 is recommended as a control antibody for monitoring of PFKFB4 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 PFKFB4 gene expression knockdown using RT-PCR Primer: PFKFB4 (h)-PR: sc-78392-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.