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SANTA CRUZ BIOTECHNOLOGY, INC.

PFK-2 br/pl siRNA (h): sc-44011

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

Phosphofructosekinase-2 (PFK-2) belongs to the phosphoglycerate mutase family and is required for the activation of cellular glycolysis. Within the glycolysis pathway, PFK-2 regulates the synthesis and degradation of fructose 2,6-bisphosphate (F2,6BP) by enzymatically catalyzing the phosphorylation of fructose-6-phosphate to form F2,6BP. F2,6BP functions as a potent activator for 6-phosphofructo-1-kinase that can then activate the glycolysis pathway. Various tissue-specific isoforms of PFK-2 are expressed, including the PFK-2 specific to the brain (br), the liver (liv) and the placenta (pl), and they are also differentially regulated and function as homodimers. A unique isoform, iPFK-2, is induced following proinflammatory stimuli, and it is also constituitively expressed in a variety of carcinoma cell lines, where it leads to an accumation of intracellular F2,6BP. In addition, the expression of iPFK-2 correlates to increases in DNA sysnthesis, suggesting that iPFK-2 may contribute to cellular transformation of cells and enhanced cellular proliferation.

REFERENCES

1. Bruni, P., et al. 1983. Increase of the glycolytic rate in human resting fibro-blasts following serum stimulation. The possible role of the fructose-2,6-bisphosphate. FEBS Lett. 159: 39-42.
2. Algaier, J., et al. 1988. Molecular cloning, sequence analysis, and expression of a human liver cDNA coding for fructose-6-P,2-kinase/fructose-2,6-bisphosphatase. Biochem. Biophys. Res. Commun. 153: 328-333.
3. Cifuentes, M.E., et al. 1991. Hormonal control of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase gene expression in rat hepatoma cells. J. Biol. Chem. 266: 1557-1563.

CHROMOSOMAL LOCATION

Genetic locus: PFKFB3 (human) mapping to 10p15.1.

PRODUCT

PFK-2 br/pl 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 PFK-2 br/pl shRNA Plasmid (h): sc-44011-SH and PFK-2 br/pl shRNA (h) Lentiviral Particles: sc-44011-V as alternate gene silencing products.

For independent verification of PFK-2 br/pl (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-44011A, sc-44011B and sc-44011C.

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 br/pl siRNA (h) is recommended for the inhibition of PFK-2 br/pl 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

PFK-2 br/pl (3F3): sc-293477 is recommended as a control antibody for monitoring of PFK-2 br/pl 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 br/pl gene expression knockdown using RT-PCR Primer: PFK-2 br/pl (h)-PR: sc-44011-PR (20 µl, 554 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Lu, Q., et al. 2015. Akt inhibition attenuates rasfonin-induced autophagy and apoptosis through the glycolytic pathway in renal cancer cells. Cell Death Dis. 6: e2005.
2. Yan, S., et al. 2017. 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase isoform 3 spatially mediates autophagy through the AMPK signaling pathway. Oncotarget 8: 80909-80922.

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.