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liver FBPase shRNA (h) Lentiviral Particles: sc-45235-V

BACKGROUND

Fructose-1,6-bisphosphatase (FBPase) mediates the key reaction of carbohydrate metabolism. It catalyzes the splitting of fructose-1,6-bisphosphate into fructose 6-phosphate and inorganic phosphate. FBPase is encoded by two genes, FBP1 and FBP2, which express the liver and muscle isoforms, respectively. FBPase appears to be present in all living organisms and is regulated by AMP inhibition in most species. Inhibition of FBPase by AMP affects the turnover of bound substrate and not its affinity for substrate. The liver FBPase isoform is composed of four identical subunits. Mutations in the FBP1 gene are inherited as an autosomal recessive disorder that leads to a deficiency of FBPase, which is associated with hypoglycemia and metabolic acidosis. Muscle FBPase is located on both sides of the z-line.

REFERENCES

1. Dzugaj, A., et al. 1980. Purification of human liver fructose-1,6-bisphosphatase. *Biochim. Biophys. Acta* 614: 407-412.
2. Marcus, F., et al. 1987. Function, structure and evolution of fructose-1,6-bisphosphatase. *Arch. Biol. Med. Exp.* 20: 371-378.
3. Matsuura, T., et al. 2002. Two newly identified genomic mutations in a Japanese female patient with fructose-1,6-bisphosphatase (FBPase) deficiency. *Mol. Genet. Metab.* 76: 207-210.
4. Rakus, D., et al. 2003. Different sensitivities of mutants and chimeric forms of human muscle and liver fructose-1,6-bisphosphatases towards AMP. *Biol. Chem.* 384: 51-58.
5. Rakus, D., et al. 2004. Interaction between muscle aldolase and muscle fructose 1,6-bisphosphatase results in the substrate channeling. *Biochemistry* 43: 14948-14957.

CHROMOSOMAL LOCATION

Genetic locus: FBP1 (human) mapping to 9q22.32.

PRODUCT

liver FBPase shRNA (h) 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 liver FBPase siRNA (h): sc-45235 and liver FBPase shRNA Plasmid (h): sc-45235-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

liver FBPase shRNA (h) Lentiviral Particles is recommended for the inhibition of liver FBPase expression in human 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

liver FBPase (B-11): sc-376388 is recommended as a control antibody for monitoring of liver FBPase 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 liver FBPase gene expression knockdown using RT-PCR Primer: liver FBPase (h)-PR: sc-45235-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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.