

muscle FBPase siRNA (m): sc-45240

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 is 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.
6. Gizak, A., et al. 2005. Nuclear localization of fructose 1,6-bisphosphatase in smooth muscle cells. *J. Mol. Histol.* 36: 243-248.

CHROMOSOMAL LOCATION

Genetic locus: Fbp2 (mouse) mapping to 13 B3.

PRODUCT

muscle FBPase 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see muscle FBPase shRNA Plasmid (m): sc-45240-SH and muscle FBPase shRNA (m) Lentiviral Particles: sc-45240-V as alternate gene silencing products.

For independent verification of muscle FBPase (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-45240A, sc-45240B and sc-45240C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

muscle FBPase siRNA (m) is recommended for the inhibition of muscle FBPase 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 μ M in 66 μ l. 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

muscle FBPase (E-11): sc-390209 is recommended as a control antibody for monitoring of muscle 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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 muscle FBPase gene expression knockdown using RT-PCR Primer: muscle FBPase (m)-PR: sc-45240-PR (20 μ l, 485 bp). 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.