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PEPCK-M shRNA (h) Lentiviral Particles: sc-44912-V

BACKGROUND

Normal adjustment to changes in blood glucose levels depends on Insulin signaling as well as enzymes involved in the regulation of gluconeogenesis. Pathological changes to this process are central to the type 2 diabetes phenotype. Phosphoenolpyruvate carboxykinase (PEPCK) plays an important role in this process by stimulating hepatic glucose production. PEPCK expression increases in response to glucagon and glucocorticoids, while Insulin suppresses expression. Modulation of the signals governing PEPCK levels present a potential therapeutic approach to the treatment of Insulin resistance and consequently obesity. The cytosolic form of PEPCK, known as PEPCK-C, and the mitochondrial form, known as PEPCK-M, are encoded by two different nuclear genes in mouse, human and chicken.

REFERENCES

1. Beale, E.G., et al. 1986. Insulin decreases H4IIE cell PEPCK mRNA by a mechanism that does not involve cAMP. *Diabetes* 35: 546-549.
2. O'Brien, R.M., et al. 1990. Identification of a sequence in the PEPCK gene that mediates a negative effect of Insulin on transcription. *Science* 249: 533-537.
3. Wang, Y. and Taub, M. 1991. Insulin and other regulatory factors modulate the growth and the phosphoenolpyruvate carboxykinase (PEPCK) activity of primary rabbit kidney proximal tubule cells in serum free medium. *J. Cell. Physiol.* 147: 374-382.
4. Barthel, A., et al. 2003. Novel concepts in Insulin regulation of hepatic gluconeogenesis. *Am. J. Physiol. Endocrinol. Metab.* 285: 685-692.
5. Horikawa, Y., et al. 2003. Identification of a novel variant in the phosphoenolpyruvate carboxykinase gene promoter in Japanese patients with type 2 diabetes. *Horm. Metab. Res.* 35: 308-312.

CHROMOSOMAL LOCATION

Genetic locus: PCK2 (human) mapping to 14q11.2.

PRODUCT

PEPCK-M 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 PEPCK-M siRNA (h): sc-44912 and PEPCK-M shRNA Plasmid (h): sc-44912-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

PEPCK-M shRNA (h) Lentiviral Particles is recommended for the inhibition of PEPCK-M 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

PEPCK-M (C-21): sc-130225 is recommended as a control antibody for monitoring of PEPCK-M 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PEPCK-M gene expression knockdown using RT-PCR Primer: PEPCK-M (h)-PR: sc-44912-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.