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SANTA CRUZ BIOTECHNOLOGY, INC.

PEPCK-M siRNA (m): sc-152164

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.
6. Barthel, A., et al. 2003. Novel aspects in the mechanisms of steroid diabetes and the regulation of hepatic glucose production by Insulin and steroids. Med. Klin. 98: 283-286.
7. Shklyaev, S., et al. 2003. Sustained peripheral expression of transgene adiponectin offsets the development of diet-induced obesity in rats. Proc. Natl. Acad. Sci. USA 100: 14217-14222.

CHROMOSOMAL LOCATION

Genetic locus: Pck2 (mouse) mapping to 14 C3.

PRODUCT

PEPCK-M 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PEPCK-M shRNA Plasmid (m): sc-152164-SH and PEPCK-M shRNA (m) Lentiviral Particles: sc-152164-V as alternate gene silencing products.

For independent verification of PEPCK-M (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-152164A, sc-152164B and sc-152164C.

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

PEPCK-M siRNA (m) is recommended for the inhibition of PEPCK-M 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 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

PEPCK (F-3): sc-271029 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 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 PEPCK-M gene expression knockdown using RT-PCR Primer: PEPCK-M (m)-PR: sc-152164-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.