SANTA CRUZ BIOTECHNOLOGY, INC.

PDPc siRNA (h): sc-77635

BACKGROUND

Pyruvate dehydrogenase phosphatase (PDP) is a serine phosphatase that catalyzes the dephosphorylation and reactivation of the α subunit of the E1 component of the mitochondrial pyruvate dehydrogenase multienzyme complex. PDP is a heterodimer that consists of catalytic and regulatory subunits. PDPc (pyruvate dehydrogenase phosphatase, catalytic subunit 1), also known as Protein phosphatase 2C, is a 537 amino acid protein that is localized within the mitochondrial matrix. PDPc is stimulated by calcium binding and utilizes two magnesium ions as cofactors. PDPc efficiently dephosphorylates all three phosphorylation sites located on the α chain of the E1 component, which simultaneously activates pyruvate dehydrogenase to convert pyruvate to acetyl-CoA for utilization in the Kreb's Cycle. Defects in the gene encoding PDPc are the cause of pyruvate dehydrogenase phosphatase deficiency, which results in lactic acidosis and neurological dysfunction.

REFERENCES

1. Hu, R.M., et al. 2000. Gene expression profiling in the human hypothalamus-pituitary-adrenal axis and full-length cDNA cloning. Proc. Natl. Acad. Sci. USA 97: 9543-9548.
2. Patel, M.S., et al. 2001. Regulation of mammalian pyruvate dehydrogenase complex by phosphorylation: complexity of multiple phosphorylation sites and kinases. Exp. Mol. Med. 33: 191-197.
3. Karpova, T., et al. 2003. Characterization of the isozymes of pyruvate dehydrogenase phosphatase: implications for the regulation of pyruvate dehydrogenase activity. Biochim. Biophys. Acta 1652: 126-135.
4. Karpova, T., et al. 2004. Probing a putative active site of the catalytic subunit of pyruvate dehydrogenase phosphatase 1 (PDP1c) by site-directed mutagenesis. Biochim. Biophys. Acta 1700: 43-51.
5. Maj, M.C., et al. 2005. Pyruvate dehydrogenase phosphatase deficiency: identification of the first mutation in two brothers and restoration of activity by protein complementation. J. Clin. Endocrinol. Metab. 90: 4101-4107.

CHROMOSOMAL LOCATION

Genetic locus: PDP1 (human) mapping to 8q22.1.

PRODUCT

PDPc 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 PDPc shRNA Plasmid (h): sc-77635-SH and PDPc shRNA (h) Lentiviral Particles: sc-77635-V as alternate gene silencing products.

For independent verification of PDPc (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-77635A, sc-77635B and sc-77635C.

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 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

PDPc siRNA (h) is recommended for the inhibition of PDPc 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

PDPc (D-11): sc-398117 is recommended as a control antibody for monitoring of PDPc 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 PDPc gene expression knockdown using RT-PCR Primer: PDPc (h)-PR: sc-77635-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.