SANTA CRUZ BIOTECHNOLOGY, INC.

PDK1 siRNA (m): sc-36204

BACKGROUND

Mitochondrial pyruvate dehydrogenase (PDH) catalyzes the oxidative decarboxylation of pyruvate and plays a central role in the regulation of homeostasis of carbohydrate fuels in mammals. PDH activity is controlled by a phosphorylation/dephosphorylation cycle, phosphorylation leading to inactivation and dephosphorylation leading to reactivation of PDH. The phosphorylation of PDH is catalyzed by pyruvate dehydrogenase kinase (PDK), the activity of which is stimulated by the products of PDH catalysis. PDK1 consists of α and β subunits; the kinase activity resides in the α subunit. Three PDK isoenzymes have been identified in humans (PDK1, 2 and 3) and two have been identified in rodent (PDK1 and 2).

REFERENCES

1. Linn, T.C., et al. 1969. α-keto acid dehydrogenase complexes. X. Regulation of the activity of the pyruvate dehydrogenase complex from beef kidney mitochondria by phosphorylation and dephosphorylation. Proc. Natl. Acad. Sci. USA 62: 234-241.
2. Hucho, F., et al. 1972. α-keto acid dehydrogenase complexes. XVII. Kinetic and regulatory properties of pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase from bovine kidney and heart. Arch. Biochem. Biophys. 151: 328-340.
3. Cate, R.L., et al. 1978. A unifying mechanism for stimulation of mammalian pyruvate dehydrogenase$_a$ kinase by reduced nicotinamide adenine dinucleotide, dihydrolipamide, acetyl coenzyme A, or pyruvate. J. Biol. Chem. 253: 496-503.
4. Stepp, L.R., et al. 1983. Purification and properties of pyruvate dehydrogenase kinase from bovine kidney. J. Biol. Chem. 258: 9454-9458.

CHROMOSOMAL LOCATION

Genetic locus: Pdk1 (mouse) mapping to 2 C3.

PRODUCT

PDK1 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 PDK1 shRNA Plasmid (m): sc-36204-SH and PDK1 shRNA (m) Lentiviral Particles: sc-36204-V as alternate gene silencing products.

For independent verification of PDK1 (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-36204A, sc-36204B and sc-36204C.

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

PDK1 siRNA (m) is recommended for the inhibition of PDK1 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

PDK1 (E-10): sc-515944 is recommended as a control antibody for monitoring of PDK1 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 PDK1 gene expression knockdown using RT-PCR Primer: PDK1 (m)-PR: sc-36204-PR (20 µl, 599 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Nishizaki, T., et al. 2016. The phosphatidylethanolamine derivative diDCP-LA-PE mimics intracellular insulin signaling. Sci. Rep. 6: 27267.
2. Na, Y.R., et al. 2020. Pyruvate dehydrogenase kinase is a negative regulator of interleukin-10 production in macrophages. J. Mol. Cell Biol. 12: 543-555.

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.