

PDK3 siRNA (h): sc-39029

BACKGROUND

Pyruvate dehydrogenase kinase family members (PDK1, 2, 3, 4) are serine kinases that catalyze phosphorylation of the E1 α subunit of the pyruvate dehydrogenase complex (PDC). PDC activity is controlled through phosphorylation and dephosphorylation of the E1 α subunit, which leads to inactivation and reactivation, respectively. PDK3 binding to a free lipoyl domain (L2) in dihydrolipoyl acetyltransferase (E2), which comprises the core of PDC, leads to a large increase in E1 α phosphorylation. Upregulation of PDK isoenzymes occurs during starvation conditions, where acetyl-CoA is alternatively generated through fatty acid oxidation. PDKs contain five conserved regions and are mechanistically similar to bacterial His-kinases in that both require histidine residues for activity. In mammals, transcripts for PDK3 are most abundant in testis and moderately expressed in heart and skeletal muscle.

REFERENCES

1. Gudi, R., et al. 1995. Diversity of the pyruvate dehydrogenase kinase gene family in humans. *J. Biol. Chem.* 270: 28989-28994.
2. Bowker-Kinley, M.M., et al. 1998. Evidence for existence of tissue-specific regulation of the mammalian pyruvate dehydrogenase complex. *Biochem. J.* 329: 191-196.
3. Sugden, M.C., et al. 2000. Selective modification of the pyruvate dehydrogenase kinase isoform profile in skeletal muscle in hyperthyroidism: implications for the regulatory impact of glucose on fatty acid oxidation. *J. Endocrinol.* 167: 339-345.
4. Mooney, B.P., et al. 2000. Histidine modifying agents abolish pyruvate dehydrogenase kinase activity. *Biochem. Biophys. Res. Commun.* 267: 500-503.

CHROMOSOMAL LOCATION

Genetic locus: PDK3 (human) mapping to Xp22.11.

PRODUCT

PDK3 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PDK3 shRNA Plasmid (h): sc-39029-SH and PDK3 shRNA (h) Lentiviral Particles: sc-39029-V as alternate gene silencing products.

For independent verification of PDK3 (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-39029A, sc-39029B and sc-39029C.

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

PDK3 siRNA (h) is recommended for the inhibition of PDK3 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 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

PDK3 (A-4): sc-365378 is recommended as a control antibody for monitoring of PDK3 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 MarkerTM 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 PDK3 gene expression knockdown using RT-PCR Primer: PDK3 (h)-PR: sc-39029-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kluz, J., et al. 2012. Inactivation of the HIF-1 α /PDK3 signaling axis drives melanoma toward mitochondrial oxidative metabolism and potentiates the therapeutic activity of pro-oxidants. *Cancer Res.* 72: 5035-5047.

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.