

PDPK1 siRNA (h): sc-29448

BACKGROUND

PDPK1 (3-phosphoinositide dependent protein kinase 1), also known as PDK1, PDPK2, PDPK2P or PR00461, is 556 amino acid ubiquitously expressed protein that localizes to the cell membrane, cytoplasm and nucleus. Acting as a master kinase, PDPK1 phosphorylates and activates a subgroup of the AGC family of protein kinases. PDPK1 is involved in mediating signal transduction for controlling proliferation, survival, and growth of developing pancreatic beta cells, regulating Ca^{2+} uptake and Ca^{2+} -activated K^{+} channels of mast cells, regulation of chemotaxis and motility of vascular endothelial cells, cardiac homeostasis, and thymocyte development. Belonging to the protein kinase superfamily, PDPK1 contains a PH domain, which play an essential role in homodimerization, localization and nuclear import of PDPK1, and a protein kinase domain. PDPK1 exists as five alternatively spliced isoforms and is encoded by a gene located on human chromosome 16p13.3.

REFERENCES

1. Alessi, D.R., et al. 1997. 3-phosphoinositide-dependent protein kinase-1 (PDK1): structural and functional homology with the *Drosophila* DSTPK61 kinase. *Curr. Biol.* 7: 776-789.
2. Stephens, L., et al. 1998. Protein kinase B kinases that mediate phosphatidylinositol 3,4,5-trisphosphate-dependent activation of protein kinase B. *Science* 279: 710-714.
3. Mora, A., et al. 2004. PDK1, the master regulator of AGC kinase signal transduction. *Semin. Cell Dev. Biol.* 15: 161-170.
4. Feldman, R.I., et al. 2005. Novel small molecule inhibitors of 3-phosphoinositide-dependent kinase-1. *J. Biol. Chem.* 280: 19867-19874.

CHROMOSOMAL LOCATION

Genetic locus: PDPK1 (human) mapping to 16p13.3.

PRODUCT

PDPK1 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 PDPK1 shRNA Plasmid (h): sc-29448-SH and PDPK1 shRNA (h) Lentiviral Particles: sc-29448-V as alternate gene silencing products.

For independent verification of PDPK1 (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-29448A, sc-29448B and sc-29448C.

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

PDPK1 siRNA (h) is recommended for the inhibition of PDPK1 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

PDPK1 (E-3): sc-17765 is recommended as a control antibody for monitoring of PDPK1 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 Marker[™] 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 PDPK1 gene expression knockdown using RT-PCR Primer: PDPK1 (h)-PR: sc-29448-PR (20 μl , 509 bp). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

SELECT PRODUCT CITATIONS

1. Bamodu, O.A., et al. 2020. Elevated PDK1 expression drives PI3K/Akt/MTOR signaling promotes radiation-resistant and dedifferentiated phenotype of hepatocellular carcinoma. *Cells* 9 pii: E746.

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.