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PP2C κ siRNA (m): sc-155944

BACKGROUND

PP2C κ (protein phosphatase 2C, isoform κ), also known as PP2C-type mitochondrial phosphoprotein phosphatase and PP2C domain-containing protein phosphatase 1K, is a 372 amino acid mitochondrial matrix protein that regulates the opening of mitochondrial membrane permeability transition pores. PP2C κ is essential for cell survival, cardiac function and embryonic development. Knockdown of PP2C κ results in cell death due to loss of mitochondrial membrane potential. PP2C κ specifically binds to the branched-chain- α -ketoacid dehydrogenase (BCKD) complex and induces dephosphorylation of Ser 293, effectively leading to the inhibition of branched chain amino acid metabolism. Highest expression of PP2C κ is found in brain, dia-phragm and heart. There are three isoforms of PP2C that are produced as a result of alternative splicing events.

REFERENCES

1. Brautigan, D.L. 1997. Phosphatases as partners in signaling networks. *Adv. Second Messenger Phosphoprotein Res.* 31: 113-124.
2. Ruiz-Meana, M., et al. 2007. Opening of mitochondrial permeability transition pore induces hypercontracture in Ca²⁺ overloaded cardiac myocytes. *Basic Res. Cardiol.* 102: 542-552.
3. Joshi, M., et al. 2007. Identification of a novel PP2C-type mitochondrial phosphatase. *Biochem. Biophys. Res. Commun.* 356: 38-44.
4. Javadov, S., et al. 2007. Mitochondrial permeability transition pore opening as an endpoint to initiate cell death and as a putative target for cardioprotection. *Cell. Physiol. Biochem.* 20: 1-22.
5. Lu, G., et al. 2007. A novel mitochondrial matrix serine/threonine protein phosphatase regulates the mitochondria permeability transition pore and is essential for cellular survival and development. *Genes Dev.* 21: 784-796.
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CHROMOSOMAL LOCATION

Genetic locus: Ppm1k (mouse) mapping to 6 B3.

PRODUCT

PP2C κ 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PP2C κ shRNA Plasmid (m): sc-155944-SH and PP2C κ shRNA (m) Lentiviral Particles: sc-155944-V as alternate gene silencing products.

For independent verification of PP2C κ (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-155944A, sc-155944B and sc-155944C.

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 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

PP2C κ siRNA (m) is recommended for the inhibition of PP2C κ 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 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

PP2C κ (E-3): sc-514924 is recommended as a control antibody for monitoring of PP2C κ 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 PP2C κ gene expression knockdown using RT-PCR Primer: PP2C κ (m)-PR: sc-155944-PR (20 μ l). 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.