

PP2C α siRNA(m): sc-45215

BACKGROUND

The phosphorylation and dephosphorylation of proteins on serine and threonine residues is an essential means of regulating a broad range of cellular functions in eukaryotes, including division, homeostasis and apoptosis. A group of proteins that are intimately involved in this process are the serine/threonine protein phosphatases. Protein phosphatase 2C α (PP2C α) has broad specificity. It dephosphorylates and negatively regulates the activities of MAP kinases and MAP kinase-kinases while also inhibiting the activation of p38 and JNK kinase cascades, induced by environmental stresses. PP2C α also induces the expression of endogenous p53 and the p53-responsive gene p21, leading to cell cycle arrest and apoptosis. The PP2C α protein, which contains an active site containing a dinuclear metal ion center, shows highest expression in epithelial cells, as well as in the digestive tract, lung, kidney, breast, prostate, endocrine glands and brain.

REFERENCES

1. Ueki, K., et al. 1992. Structure and expression of two isoforms of the murine calmodulin-dependent protein phosphatase regulatory subunit (calcineurin B). *Biochem. Biophys. Res. Commun.* 187: 537-543.
2. Cohen, P.T. 1993. Important roles for novel protein phosphatases dephosphorylating serine and threonine residues. *Biochem. Soc. Trans.* 21: 884-888.
3. Yokoyama, N., et al. 1996. Purification and characterization of protein phosphatase 2C in rat parotid acinar cells: two forms of Mg²⁺-activated histone phosphatase and phosphorylation by cAMP-dependent protein kinase. *Arch. Biochem. Biophys.* 331: 1-8.
4. Takekawa, M., et al. 1998. Protein phosphatase 2C α inhibits the human stress-responsive p38 and JNK MAPK pathways. *EMBO J.* 17: 4744-4752.
5. Lifschitz-Mercer, B., et al. 2001. Protein phosphatase 2C α expression in normal human tissues: an immunohistochemical study. *Histochem. Cell Biol.* 116: 31-39.
6. Jackson, M.D., et al. 2003. Probing the function of conserved residues in the serine/threonine phosphatase PP2C α . *Biochemistry* 42: 8513-8521.
7. Ofek, P., et al. 2003. Cell cycle regulation and p53 activation by protein phosphatase 2C α . *J. Biol. Chem.* 278: 14299-14305.

CHROMOSOMAL LOCATION

Genetic locus: Ppm1a (mouse) mapping to 12 C3.

PRODUCT

PP2C α 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 α shRNA Plasmid (m): sc-45215-SH and PP2C α shRNA (m) Lentiviral Particles: sc-45215-V as alternate gene silencing products.

For independent verification of PP2C α (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-45215A, sc-45215B and sc-45215C.

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 α siRNA (m) is recommended for the inhibition of PP2C α 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 α (7F12): sc-517264 is recommended as a control antibody for monitoring of PP2C α 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 α gene expression knockdown using RT-PCR Primer: PP2C α (m)-PR: sc-45215-PR (20 μ l, 545 bp). 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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.