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PP2C α shRNA (m) Lentiviral Particles: sc-45215-V

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 and also inhibits 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: 4744-52.

CHROMOSOMAL LOCATION

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

PRODUCT

PP2C α shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0 x 10⁶ infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see PP2C α siRNA (m): sc-45215 and PP2C α shRNA Plasmid (m): sc-45215-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

PP2C α shRNA (m) Lentiviral Particles is recommended for the inhibition of PP2C α 1 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0 x 10⁶ infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

PP2C α (L-18): sc-32402 is recommended as a control antibody for monitoring of PP2C α 1 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PP2C α 1 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.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.