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I2PP2A shRNA (h) Lentiviral Particles: sc-43856-V

BACKGROUND

Protein phosphatase 2A (PP2A) is a major mammalian protein serine/threonine phosphatase that regulates diverse cellular processes. Inhibitor 1 of PP2A (I1PP2A) and inhibitor 2 of PP2A (I2PP2A), which share large sequence similarity, are heat-stable protein inhibitors of the cellular phosphatase activity of PP2A. I1PP2A and I2PP2A were initially characterized as putative HLA class II associated proteins Phap I and Phap II. These inhibitor proteins act noncompetitively to selectively inhibit PP2A, but do not affect the phosphatase activity of the related proteins PP1, PP2B and PP2C. The I1PP2A protein is localized to both the cytoplasm and the nucleus. In contrast, I2PP2A is located predominantly in the nucleus and is highly expressed in Wilms' tumor cells. Transient expression of I2PP2A in HEK-293 cells leads to an increase in the DNA binding activity of the proto-oncogene c-Jun.

REFERENCES

- Guo, H., et al. 1993. Autophosphorylation-activated protein kinase phosphorylates and inactivates protein phosphatase 2A. *Proc. Natl. Acad. Sci. USA* 90: 2500-2504.
- Damuni, Z., et al. 1994. Autophosphorylation-activated protein kinase inactivates the protein tyrosine phosphatase activity of protein phosphatase 2A. *FEBS Lett.* 352: 311-314.
- Li, M., et al. 1995. Purification and characterization of two potent heat-stable protein inhibitors of protein phosphatase 2A from bovine kidney. *Biochemistry* 34: 1988-1996.
- Li, M., et al. 1996. Molecular identification of I1PP2A, a novel potent heat-stable inhibitor protein of protein phosphatase 2A. *Biochemistry* 35: 6998-7002.
- Li, M., et al. 1996. The myeloid leukemia-associated protein SET is a potent inhibitor of protein phosphatase 2A. *J. Biol. Chem.* 271: 11059-11062.

CHROMOSOMAL LOCATION

Genetic locus: SET (human) mapping to 9q34.11.

PRODUCT

I2PP2A shRNA (h) Lentiviral Particles are concentrated, transduction-ready viral particles containing a target-specific construct that encodes a 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 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 I2PP2A siRNA (h): sc-43856 and I2PP2A shRNA Plasmid (h): sc-43856-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

I2PP2A shRNA (h) Lentiviral Particles is recommended for the inhibition of I2PP2A expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

I2PP2A (F-9): sc-133138 is recommended as a control antibody for monitoring of I2PP2A 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 goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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 goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor I2PP2A gene expression knockdown using RT-PCR Primer: I2PP2A (h)-PR: sc-43856-PR (20 μ l, 569 bp). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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.