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pan PP1 shRNA (h) Lentiviral Particles: sc-43545-V

BACKGROUND

Several major protein serine/threonine phosphatases have been identified in eukaryotic cells. These include protein phosphatases 1, 2A, 2B, X and Y (PP1, PP2A, PP2B, PP2C, PPX and PPY). These enzymes can be distinguished by their action on phosphorylase kinase and their sensitivity to certain activators and inhibitors. For example, PP1 is potently inhibited by the thermostable protein inhibitors 1 and 2 and dephosphorylates the β subunit of phosphorylase kinase specifically, whereas type 2 protein phosphatases are unaffected by these inhibitor proteins and dephosphorylate the α subunit of phosphorylase kinase preferentially. The best studied member of this family, PP2A, consists of heterotrimeric complex composed of a catalytic subunit (C) and two associated regulatory subunits (A and B). It has been shown that transient expression of SV40 small T antigen in CV-1 cells activated both MAP kinase and MEK-1 activity, and stimulated cell growth in the absence of any effect on upstream Raf-1 activity. This effect was due to the interaction between SV40 small T antigen and PP2A.

REFERENCES

- Ingebritsen, T.S., et al. 1983. Protein phosphatases: properties and role in cellular regulation. *Science* 221: 331-338.
- Cohen, P. 1989. The structure and regulation of protein phosphatases. *Ann. Rev. Biochem.* 58: 453-508.
- Cohen, P.T., et al. 1990. Protein serine/threonine phosphatases; an expanding family. *FEBS Lett.* 268: 355-359.
- Kamibayashi, C., et al. 1991. Subunit interactions control protein phosphatase 2A. Effects of limited proteolysis, N-ethylmaleimide, and heparin on the interaction of the B subunit. *J. Biol. Chem.* 266: 13251-13260.

PRODUCT

pan PP1 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 6 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 1.0×10^6 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 pan PP1 siRNA (h): sc-43545 and pan PP1 shRNA Plasmid (h): sc-43545-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.

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.

APPLICATIONS

pan PP1 shRNA (h) Lentiviral Particles is recommended for the inhibition of pan PP1 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

PP1 (E-9): sc-7482 is recommended as a control antibody for monitoring of pan PP1 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 pan PP1 gene expression knockdown using RT-PCR Primer: pan PP1 (h)-PR: sc-43545-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.

PROTOCOLS

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