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pan PP1 shRNA (m) Lentiviral Particles: sc-43533-V

BACKGROUND

In eukaryotes, the phosphorylation and dephosphorylation of proteins on serine and threonine residues is an essential means of regulating a broad range of cellular functions, including division, homeostasis and apoptosis. A group of proteins that are intimately involved in this process are the protein phosphatases. In general, the protein phosphatase (PP) holoenzyme is a trimeric complex composed of a regulatory subunit, a variable subunit and a catalytic subunit. Four major families of protein phosphatase catalytic subunit have been identified, designated PP1, PP2A, PP2B (calcineurin) and PP2C. An additional protein phosphatase catalytic subunit, PPX (also known as PP4) is a putative member of a novel PP family. The PP1 family is comprised of subfamily members PP1 α , PP1 β and PP1 γ , which are MgATP-dependent enzymes. PP1 inactivity is maintained through its association with the inhibitory protein NIPP-1 (nuclear inhibitor of PP1). Phosphorylation of NIPP-1 by cAMP-PK or casein kinase II results in the release of active PP1.

REFERENCES

1. Ingebritsen, T.S., et al. 1983. Protein phosphatases: properties and role in cellular regulation. *Science* 221: 331-338.
2. Cohen, P. 1989. The structure and regulation of protein phosphatases. *Ann. Rev. Biochem.* 58: 453-508.
3. Cohen, P.T., et al. 1990. Protein serine/threonine phosphatases; an expanding family. *FEBS Lett.* 268: 355-359.
4. Ruediger, R., et al. 1992. Identification of binding sites on the regulatory A subunit of protein phosphatase 2A for the catalytic C subunit and for tumor antigens of simian virus 40 and polyomavirus. *Mol. Cell. Biol.* 12: 4872-4882.

PRODUCT

pan PP1 shRNA (m) 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 (m): sc-43533 and pan PP1 shRNA Plasmid (m): sc-43533-SH as alternate gene silencing products.

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.

PROTOCOLS

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

APPLICATIONS

pan PP1 shRNA (m) Lentiviral Particles is recommended for the inhibition of PP1 α , PP1 β and PP1 γ expression in mouse 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

pan PP1 (E-9): sc-7482 is recommended as a control antibody for monitoring of PP1 α , PP1 β and PP1 γ 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 PP1 α , PP1 β and PP1 γ gene expression knockdown using RT-PCR Primer: pan PP1 (m)-PR: sc-43533-PR (20 μ l). 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.

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.