

PP1 γ siRNA (m): sc-36298

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. Cohen, P.T. 1993. Important roles for novel protein phosphatases dephosphorylating serine and threonine residues. *Biochem. Soc. Trans.* 21: 884-888.
2. Hendrix, P., et al. 1993. Structure and expression of a 72 kDa regulatory subunit of protein phosphatase 2A. Evidence for different size forms produced by alternative splicing. *J. Biol. Chem.* 268: 15267-15276.
3. Mumby, M.C. et al. 1993. Protein serine/threonine phosphatases: structure, regulation, and functions in cell growth. *Physiol. Rev.* 73: 673-699.
4. Okubo, S., et al. 1994. A regulatory subunit of smooth muscle myosin bound phosphatase. *Biochem. Biophys. Res. Commun.* 200: 429-434.

CHROMOSOMAL LOCATION

Genetic locus: Ppp1cc (mouse) mapping to 5 F.

PRODUCT

PP1 γ 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 PP1 γ shRNA Plasmid (m): sc-36298-SH and PP1 γ shRNA (m) Lentiviral Particles: sc-36298-V as alternate gene silencing products.

For independent verification of PP1 γ (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-36298A, sc-36298B and sc-36298C.

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

PP1 γ siRNA (m) is recommended for the inhibition of PP1 γ 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

PP1 γ (E-4): sc-515943 is recommended as a control antibody for monitoring of 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 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 MarkerTM 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 PP1 γ gene expression knockdown using RT-PCR Primer: PP1 γ (m)-PR: sc-36298-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Bielas, S.L., et al. 2007. Spinophilin facilitates dephosphorylation of doublecortin by PP1 to mediate microtubule bundling at the axonal wrist. *Cell* 129: 579-591.

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.