

PP2C ζ siRNA (h): sc-78774

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. The PP2C group of serine/threonine phosphatases are divided into subclasses according to their requirement for magnesium substrate, their structure and by insensitivity to okadaic acid. PP2C ζ (protein phosphatase 2C isoform ζ), also known as PPM1J (protein phosphatase, Mg²⁺/Mn²⁺ dependent, 1J), is a 505 amino acid phosphoprotein that contains one PP2C-like domain and belongs to the PP2C family. Two isoforms of PP2C ζ are produced as a result of alternative splicing events. PP2C ζ likely exhibits its specific role through its small ubiquitin-related modifier-1-induced recruitment to UBC9 (ubiquitin conjugating enzyme 9).

REFERENCES

- Cohen, P. and Cohen, P.T. 1989. Protein phosphatases come of age. *J. Biol. Chem.* 264: 21435-21438.
- Hanada, M., et al. 1998. Selective suppression of stress-activated protein kinase pathway by protein phosphatase 2C in mammalian cells. *FEBS Lett.* 437: 172-176.
- Zolnierowicz, S. 2000. Type 2A protein phosphatase, the complex regulator of numerous signaling pathways. *Biochem. Pharmacol.* 60: 1225-1235.
- Hanada, M., et al. 2001. Regulation of the TAK1 signaling pathway by protein phosphatase 2C. *J. Biol. Chem.* 276: 5753-5759.
- Komaki, K., et al. 2003. Molecular cloning of PP2C η , a novel member of the protein phosphatase 2C family. *Biochim. Biophys. Acta* 1630: 130-137.
- Kashiwaba, M., et al. 2003. A novel protein phosphatase 2C family member (PP2C ζ) is able to associate with ubiquitin conjugating enzyme 9. *FEBS Lett.* 538: 197-202.
- Hearnes, J.M., et al. 2005. Chromatin immunoprecipitation-based screen to identify functional genomic binding sites for sequence-specific transactivators. *Mol. Cell. Biol.* 25: 10148-10158.

CHROMOSOMAL LOCATION

Genetic locus: PPM1J (human) mapping to 1p13.2.

PRODUCT

PP2C ζ siRNA (h) 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 PP2C ζ shRNA Plasmid (h): sc-78774-SH and PP2C ζ shRNA (h) Lentiviral Particles: sc-78774-V as alternate gene silencing products.

For independent verification of PP2C ζ (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-78774A, sc-78774B and sc-78774C.

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

PP2C ζ siRNA (h) is recommended for the inhibition of PP2C ζ expression in human 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

PP2C ζ (C-4): sc-390214 is recommended as a control antibody for monitoring of PP2C ζ 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 Marker[™] 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 PP2C ζ gene expression knockdown using RT-PCR Primer: PP2C ζ (h)-PR: sc-78774-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.