SANTA CRUZ BIOTECHNOLOGY, INC.

PP2Cζ siRNA (m): sc-155945

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. PP2Cζ (protein phosphatase 2C isoform ζ), also known as PPM1J (protein phosphatase, Mg^{2+}/Mn^{2+} dependent, 1J), is a 505 amino acid phosphoprotein that contains one PP2C-like domain and belongs to the PP2C family. Two isoforms of PP2Cζ are produced as a result of alternative splicing events. PP2Cζ likely exhibits its specific role through its small ubiquitin-related modifier-1-induced recruitment to UBC9 (ubiquitin conjugating enzyme 9).

REFERENCES

1. Cohen, P. and Cohen, P.T. 1989. Protein phosphatases come of age. J. Biol. Chem. 264: 21435-21438.
2. 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.
3. Zolnierowicz, S. 2000. Type 2A protein phosphatase, the complex regulator of numerous signaling pathways. Biochem. Pharmacol. 60: 1225-1235.
4. Hanada, M., et al. 2001. Regulation of the TAK1 signaling pathway by protein phosphatase 2C. J. Biol. Chem. 276: 5753-5759.
5. Komaki, K., et al. 2003. Molecular cloning of PP2Cη, a novel member of the protein phosphatase 2C family. Biochim. Biophys. Acta 1630: 130-137.
6. Kashiwaba, M., et al. 2003. A novel protein phosphatase 2C family member (PP2Cζ) is able to associate with ubiquitin conjugating enzyme 9. FEBS Lett. 538: 197-202.
7. 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 (mouse) mapping to 3 F2.2.

PRODUCT

PP2Cζ siRNA (m) is a pool of 2 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PP2Cζ shRNA Plasmid (m): sc-155945-SH and PP2Cζ shRNA (m) Lentiviral Particles: sc-155945-V as alternate gene silencing products.

For independent verification of PP2Cζ (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-155945A and sc-155945B.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

PP2Cζ siRNA (m) is recommended for the inhibition of PP2Cζ 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 µM in 66 µl. 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

PP2Cζ (C-4): sc-390214 is recommended as a control antibody for monitoring of PP2Cζ 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 PP2Cζ gene expression knockdown using RT-PCR Primer: PP2Cζ (m)-PR: sc-155945-PR (20 µl). 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.