SANTA CRUZ BIOTECHNOLOGY, INC.

CPI-17 siRNA (h): sc-40423

BACKGROUND

CPI-17 is a phosphorylation-dependent inhibitory protein for smooth muscle Myosin phosphate. CPI-17 was originally identified as a PKC-potentiated inhibitory protein of protein phosphatase-1, which is dominantly expressed in smooth muscle. Phosphorylation at Threonine 38, *in vitro,* by PKC or Rho-kinase enhances the inhibitory potency toward Myosin phosphatase. CPI-17 is also phosphorylated at Threonine 38 by protein kinase N and might be involved in the calcium sensitization of smooth muscle contraction as a downstream effector of Rho and/or arachidonic acid. CPI-17 is dually phosphorylated at Serine 12 and Threonine 38 by a MYPT-associated kinase, M110 kinase.

REFERENCES

1. Senba, S., et al. 1999. Identification of trimeric Myosin phosphatase (PP1M) as a target for a novel PKC-potentiated protein phosphatase-1 inhibitory protein (CPI-17) in porcine aorta smooth muscle. J. Biochem. 125: 354-362.
2. Koyama, M., et al. 2000. Phosphorylation of CPI-17, an inhibitory phosphoprotein of smooth muscle Myosin phosphatase, by Rho-kinase. FEBS Lett. 475: 197-200.
3. Eto, M., et al. 2000. Inhibition of Myosin/Moesin phosphatase by expression of the phosphoinhibitor protein CPI-17 alters microfilament organization and retards cell spreading. Cell Motil. Cytoskeleton 46: 222-234.
4. Hamaguchi, T., et al. 2000. Phosphorylation of CPI-17, an inhibitor of Myosin phosphatase, by protein kinase N. Biochem. Biophys. Res. Commun. 274: 825-830.
5. Kitazawa, T., et al. 2000. Agonists trigger G protein-mediated activation of the CPI-17 inhibitor phosphoprotein of Myosin light chain phosphatase to enhance vascular smooth muscle contractility. J. Biol. Chem. 275: 9897-9900.
6. MacDonald, J.A., et al. 2001. Dual Ser and Thr phosphorylation of CPI-17, an inhibitor of myosin phosphatase, by MYPT-associated kinase. FEBS Lett. 493: 91-94.

CHROMOSOMAL LOCATION

Genetic locus: PPP1R14A (human) mapping to 19q13.2.

PRODUCT

CPI-17 siRNA (h) is a target-specific 19-25 nt siRNA 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 CPI-17 shRNA Plasmid (h): sc-40423-SH and CPI-17 shRNA (h) Lentiviral Particles: sc-40423-V as alternate gene silencing products.

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.

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

CPI-17 siRNA (h) is recommended for the inhibition of CPI-17 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 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

CPI-17 (F-4): sc-48406 is recommended as a control antibody for monitoring of CPI-17 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 CPI-17 gene expression knockdown using RT-PCR Primer: CPI-17 (h)-PR: sc-40423-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.