SANTA CRUZ BIOTECHNOLOGY, INC.

CPI-17 siRNA (m): sc-40424

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 (mouse) mapping to 7 B1.

PRODUCT

CPI-17 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CPI-17 shRNA Plasmid (m): sc-40424-SH and CPI-17 shRNA (m) Lentiviral Particles: sc-40424-V as alternate gene silencing products.

For independent verification of CPI-17 (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-40424A, sc-40424B and sc-40424C.

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 (m) is recommended for the inhibition of CPI-17 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

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 (m)-PR: sc-40424-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.