Phospho-CPI-17 Thr38 Mouse Monoclonal Antibody (Clone AK-1F11)

Cat# CY-M1024

100 µg (1 mg/ml x 100 µL)

Clone Name	Applications	Species Cross-reactivity	Molecular Wt.	Source Isotype
AK-1F11	E, WB	H, M, R	17 kDa	Mouse IgG1

Background: Myosin phosphatase (MLCP) plays a critical regulatory role in the $Ca^{(2+)}$ sensitivity of myosin phosphorylation and smooth muscle contraction. It has been suggested that phosphorylation at Thr696 of the MLCP regulatory subunit (MBS/MYPT1) and at Thr38 of the CPI-17, which is MLCP inhibitor protein, results in inhibition of MLCP activity. The phosphorylation of CPI-17 Thr38 is thought to play an important role in G-protein-mediated inhibition of MLCP in tonic arterial smooth muscle. The CPI-17 phosphorylation transiently increased after agonist stimulation in both alpha-toxin skinned and intact fibers. The time course of the increase in CPI-17 phosphorylation after stimulation correlated with the increase in myosin regulatory light chain (MLC_{20}) phosphorylation. These results strongly suggest that the phosphorylation of CPI-17 plays a significant role in the agonist-induced increase in myosin phosphorylation and contraction of smooth muscle in the $Ca^{(2+)}$-independent activation mechanism of smooth muscle contraction.

This anti-Phospho-CPI-17 Thr38 monoclonal antibody has been validated with PKC, however it has the potential for use in evaluating other serine threonine kinases such as Rho-Kinase, protein kinase N and ILK.

Specificity/Sensitivity: Phospho-CPI-17 Thr38 Antibody detects phosphorylated recombinant CPI-17 when phosphorylated at threonine38 in vitro by means of western blotting and ELISA.

Source/Purification: Monoclonal antibody is produced by immunizing mice with a synthetic phosphopeptide corresponding to residues surrounding Thr38 of human CPI-17. IgG is purified by protein A-sepharose chromatography.

Recommended Antibody Dilutions: Western blotting: 1-2 µg/mL, ELISA for detection of PKC activity: 1 µg/mL

Storage: Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50 % glycerol. Store at -20°C.

Applications Key: WB: Western Blotting **IP:**Immunoprecipitation **IHC:**Immunohistochemistry **IC:**Immunocytochemistry **F:**Flow cytometry **E:**ELISA **FP:**Fluorescence Polarization assay
Species Cross-Reactivity Key: H:human **M:**mouse **R:**rat **Hm:**hamster **Mk:**monkey **Mi:**mink **C:**chicken **X:***Xenopus* **Z:**zebra fish **All:** all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Selected Application References:

1. Hirano K, Derkach DN, Hirano M, Nishimura J, Kanaide H. Protein kinase network in the regulation of phosphorylation and dephosphorylation of smooth muscle myosin light chain. *Mol Cell Biochem*. 2003 Jun;**248**(1-2):105-14.
2. Somlyo AP, Somlyo AV. Signal transduction by G-proteins, rho-kinase and protein phosphatase to smooth muscle and non-muscle myosin II. *J Physiol*. 2000 Jan 15;**522** Pt 2:177-85.
3. Niiro N, Koga Y, Ikebe M. Agonist-induced changes in the phosphorylation of the myosin- binding subunit of myosin light chain phosphatase and CPI17, two regulatory factors of myosin light chain phosphatase, in smooth muscle. *Biochem J*. 2003 Jan 1;**369**(Pt 1):117-28.
4. Kitazawa T, Eto M, Woodsome TP, Khalequzzaman M. Phosphorylation of the myosin phosphatase targeting subunit and CPI-17 during Ca2+ sensitization in rabbit smooth muscle. *J Physiol*. 2003 Feb 1;**546**(Pt 3):879-89.
5. Kitazawa T, Eto M, Woodsome TP, Brautigan DL. 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*. 2000 Apr 7;**275**(14):9897-900.
6. Woodsome TP, Eto M, Everett A, Brautigan DL, Kitazawa T. Expression of CPI-17 and myosin phosphatase correlates with Ca(2+) sensitivity of protein kinase C-induced contraction in rabbit smooth muscle. *J Physiol*. 2001 Sep 1;**535**(Pt 2):553-64.
7. Deng JT, Sutherland C, Brautigan DL, Eto M, Walsh MP. Phosphorylation of the myosin phosphatase inhibitors, CPI-17 and PHI-1, by integrin-linked kinase. *Biochem J*. 2002 Oct 15;**367**(Pt 2):517-24.
8. Li L, Eto M, Lee MR, Morita F, Yazawa M, Kitazawa T. Possible involvement of the novel CPI-17 protein in protein kinase C signal transduction of rabbit arterial smooth muscle. *J Physiol*. 1998 May 1;**508** (Pt 3):871-81.
9. Kitazawa T, Eto M, Woodsome TP, Brautigan DL. 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*. 2000 Apr 7;**275**(14):9897-900.

Fig.1 ELISA for measurement of recombinant PKC activity using Phospho-CPI-17 Thr38 Monoclonal antibody (AK-1F11) in CycLex PKC Assay/Drug Discovery Kit (Cat# CY-1185: Available soon)

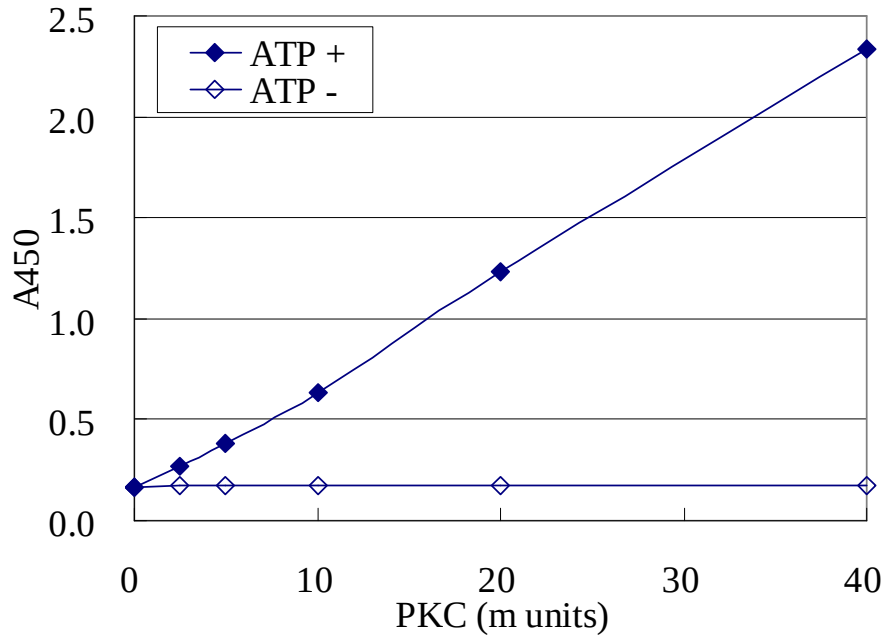
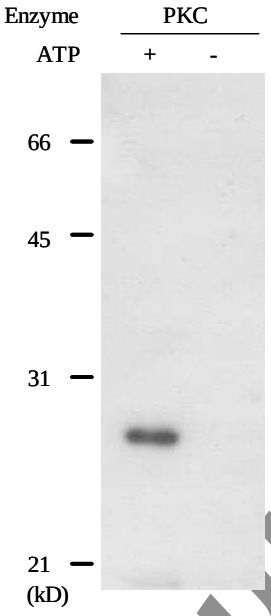


Fig.2 In vitro kinase reaction and detection of phosphorylation of CPI-17 at Thr38 residue by using Phospho-CPI-17 Thr38 Monoclonal antibody (AK-1F11). GST-CPI-17 was phosphorylated by protein kinase C derived from rat brain in vitro and proved by Phospho-CPI-17 Thr38 Monoclonal antibody (AK-1F11) after SDS-PAGE and transfer to PVDF membrane.



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