



## MYPT1 pSer445 (mouse; residues 437-452), pAb

Alternate Names: Protein Phosphatase 1, Regulatory Subunit 12A, PPP1R12A, Myosin Phosphate target subunit 1

Cat. No. 68-0043-100  
Lot. No. 30282

Quantity: 100 µg  
Storage: -20°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS

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This antibody was developed and validated by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (University of Dundee, Dundee, UK).

### Background

Protein ubiquitylation and protein phosphorylation are the two major mechanisms that regulate the functions of proteins in eukaryotic cells. However, these different post-translational modifications do not operate independently of one another, but are frequently interlinked to enable biological processes to be controlled in a more complex and sophisticated manner. Studying how protein phosphorylation events control the ubiquitin system and how ubiquitylation regulates protein phosphorylation has become a focal point of the study of cell regulation and human disease. The mammalian MYPT family consists of the products of five genes, denoted MYPT1, MYPT2, MBS85, MYPT3 and TIMAP (Grassie *et al.*, 2011). Myosin phosphatase (MP) activity, which regulates smooth muscle relaxation, is regulated by the phosphorylation of its regulatory subunit, myosin phosphatase targeting subunit 1 (MYPT1) (Cheng *et al.*, 2013). Cloning of human MYPT1 was first described by Takahashi *et al.* (1997). An example of the interplay between phosphorylation, ubiquitylation, and methylation, has been highlighted in a recent study showing that MYPT1 can be methylated *in vitro* and *in vivo* by histone lysine methyltransferase SETD7 and demethylated by histone demethylase LSD1. LSD1 silencing increased MYPT1 protein levels, decreasing the steady state level of phosphorylated retinoblastoma 1 (RB1; Ser

### Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: mouse MYPT1 (residues 437 – 452) [RLGLRKTG(pS)YGALAEI]

Purification: affinity-purified against phospho peptide

Formulation: phosphate-buffered saline

Specificity: detects MYPT1 at ~130 kDa

Reactivity: mouse; other species not tested

Stability/Storage: 12 months at -20°C; aliquot as required

### Research Applications and Quality Assurance

#### Western Immunoblotting:

use 1 µg/ml: add 10 µg of the non-phosphorylated form of the peptide immunogen (Cat# 68-1004-001 provided) to your immunoblotting incubation per 1 µg of polyclonal antibody in order to deplete any non-phospho specific polyclonal antibodies present.

#### Immunoprecipitation:

not tested



#### Western Blotting Analysis:

Following the transfection of HEK293 cells with FLAG-tagged wild-type (wt) MYPT1 or the indicated mutant versions of MYPT1 (also FLAG-tagged), cells were lysed and immunoprecipitation was performed using a commercially available anti-FLAG antibody. Western blotting was subsequently performed probing with a commercially available anti-FLAG antibody or the anti-MYPT1 pSer445 antibody (Cat# 68-0043-100). A band was detected from FLAG-tagged immunoprecipitated cell lysates except those expressing the S445A mutant and the triple mutant (SSS/AAA) when probed with 1.0 µg/ml of anti-MYPT1 pSer445 mouse polyclonal antibody (Cat# 68-0043-100).

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Lot-specific COA version tracker: v1.0.0



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## Background

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807/811) and reducing E2F activity. Demethylation of MYPT1 has been shown to increase the ubiquitin proteasome pathway-dependent turnover of MYPT1. This study offers a novel cell cycle regulatory mechanism mediated by methylation/demethylation dynamics, and also reveals the significance of LSD1 overexpression in human carcinogenesis (Cho *et al.*, 2011).

### Antibody Production:

Anti-MYPT1 pSer445 (mouse) polyclonal antibody was raised in sheep against MYPT1 (residues 437-452 of mouse MYPT1; Ser445 phosphorylated). The antibodies were purified by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (MRC-PPU, University of Dundee, Dundee, U.K.) by affinity purification of the anti-MYPT1 pAbs from the sheep serum using a GST-tagged antigen-agarose column. Anti-MYPT1 pSer445 (mouse) pAb was sourced by Ubiquigent directly from the MRC-PPU.

### General References:

Cheng JC, Cheng HP, Tsai IC and Jiang MJ (2013) ROS-mediated downregulation of MYPT1 in smooth muscle cells: a potential mechanism for the aberrant contractility in atherosclerosis. *Lab Invest* **93**, 422-433.

Cho HS, Suzuki T, Dohmae N, Hayami S, Unoki M, Yoshimatsu M, *et al.* (2011) Demethylation of RB regulator MYPT1 by histone demethylase LSD1 promotes cell cycle progression in cancer cells. *Cancer Res* **71**, 655-660.

Grassie ME, Moffat LD, Walsh MP and MacDonald JA (2011) The myosin phosphatase targeting protein (MYPT) family: a regulated mechanism for achieving substrate specificity of the catalytic subunit of protein phosphatase type 1delta. *Arch Biochem Biophys* **510**, 147-159.

Takahashi N, Ito M, Tanaka J, Nakano T, Kaibuchi K, Odai H, *et al.* (1997) Localization of the gene coding for myosin phosphatase, target subunit 1 (MYPT1) to human chromosome 12q15-q21. *Genomics* **44**, 150-152.



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