MYPT1 pSer554 (mouse; residues 547-618), pAb

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

Cat. No.	**68-0045-100**	Quantity:	100 µg
Lot. No.	**30284**	Storage:	-20˚C

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CERTIFICATE OF ANALYSIS | **Page 1 of 2**

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

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Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: mouse MYPT1 (residues 547-618) [STYHRST(pS)NRLWAEDS]

Purification: affinity-purified against phospho peptide

Formulation: phosphate-buffered saline

Specificity: detects MYPT1 at ~130 kDa

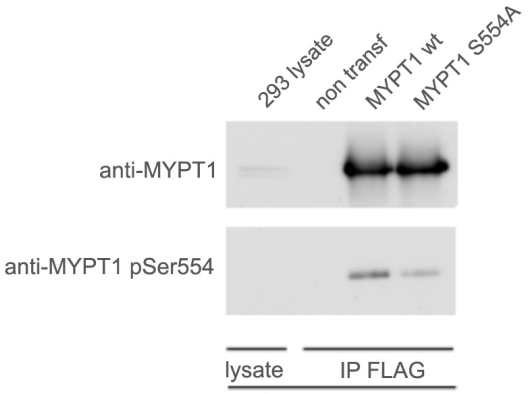
Reactivity: mouse; other species not tested

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# 68-1006-001 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 MYPT1 (MYPT1 wt) or a FLAG-tagged MYPT1 mutant (MYPT1 S554A), cells were lysed and immunoprecipitation was performed using a commercially available anti-FLAG antibody. Immunoprecipitation of untransfected HEK293 cell lysate (non transf) was also included as a control. Western blotting was subsequently performed probing with a commercially available anti-MYPT1 antibody or the anti-MYPT1 pSer554 antibody (Cat# 68-0045-100). A band was detected from the FLAG-tagged immunoprecipitate of the MYPT1 wt, and to a lesser extent the MYPT1 S554A mutant cell lysates when probed with 1.0 µg/ml of the anti MYPT1 pSer554 mouse polyclonal antibody (Cat# 68-0045-100). HEK293 cell lysate (293 lysate) was included in the Western blotting experiment as a control.

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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 pSer554 (mouse) polyclonal antibody was raised in sheep against MYPT1 (residues 547-618 of mouse MYPT1; Ser554 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 pSer554 (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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