

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 posttranslational 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 ubiguitin system and how ubiguitylation 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). Cloning of human MYPT2 was first described by Fujioka et al. (1998). MYPT2 is predominantly expressed in striated muscle and brain (Fujioka et al., 1998), MYPT1, a target subunit of myosin phosphatase (MP), has several important functions including activation and regulation of phosphatase activity. Because of similarities in structure between MYPT1 and MYPT2. MYPT2 also serves as a target subunit in MP of striated muscle and brain. (Okamoto et al., 2006). Several features of MYPT2 and MYPT1 are similar, including: a specific interaction with the catalytic subunit of type 1 phosphatase,  $\delta$  isoform (PP1c $\delta$ ); interaction

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# MYPT2 (human; residues 350-450), pAb

Alternate Names: Protein Phosphatase 1 regulatory subunit 12B, PPP1R12B, Myosin phosphatase target subunit 2

Cat. No. Lot. No.

68-0047-100 30286 Quantity: Storage: 100 µg -20°С

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## **Physical Characteristics**

**CERTIFICATE OF ANALYSIS** 

Quantity: 100 µg

**Concentration:** to be provided on shipping

Source: sheep polyclonal antibody

**Immunogen:** human, MYPT2 (residues 350-450) [GST-tagged]

**Purification:** affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

**Specificity:** detects MYPT2 at ~110 kDa

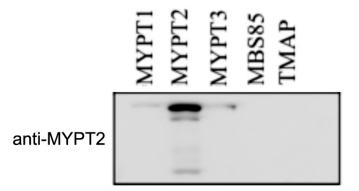
Reactivity: human; other species not tested

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

# **Research Applications and Quality Assurance**

Western Immunoblotting: use 1 µg/ml

Immunoprecipitation: not tested



#### Western Blotting Analysis:

Following the transfection of HEK293 cells with FLAG-MYPT1, FLAG-MYPT2, FLAG-MYPT3, FLAG-MBS85 and FLAG-TMAP, cells were lysed and immunoprecipitation was performed using a commercially available anti-FLAG antibody. By Western blotting, a band was detected predominantly from FLAG-tagged immunoprecipitation sample derived from the cell lysates transfected with FLAG-MYPT2 when probed with 1 µg/ml of anti-MYPT2 polyclonal antibody (Cat# 68-0047-100).

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### Background

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of MYPT2 with the small heart-specific MP subunit; interaction of the C-terminal region of MYPT2 with the active form of RhoA; phosphorylation by Rho-kinase at an inhibitory site (Okamoto et al., 2006). MYPT2 helps regulate the activity of myosin phosphatases and enhances the sensitivity of the contractile apparatus to Ca<sup>2+</sup>. As part of the protein phosphatase 1 complex, MYPT2 is responsible for binding the complex to myosin filaments (Moorhead et al., 1998). Cardiac myosin is the primary substrate targeted by the MYPT2 apoenzyme; MYPT2 and has been detected along the A-band and Zline of sarcomeres indicating it likely operates on multiple substrates (Ito et al., 2004).

### **Antibody Production:**

Anti-MYPT2 (human) polyclonal antibody was raised in sheep against MYPT2 (residues 350-450 of human MYPT2). 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-MYPT2 pAbs from the sheep serum using an antigen-agarose column followed by depletion of any anti-GST antibodies using a GST-agarose column. Anti-MYPT2 (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

#### General References:

Fujioka M, Takahashi N, Odai H, Araki S, Ichikawa K, et al. (1998) A new isoform of human myosin phosphatase targeting/regulatory subunit (MYPT2): cDNA cloning, tissue expression, and chromosomal mapping. *Genomics* **49**, 59-68.

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.

Moorhead, G. (1998) The major myosin phosphatase in skeletal muscle is a complex between the  $\beta$ -isoform of protein phosphatase 1 and the MYPT2 gene product. *FEBS Lett* **438**, 141-144.

Okamoto R, Kato T, Mizoguchi A, Takahashi N, Nakakuki T et al. (2006) Characterization and function of MYPT2, a target subunit of myosin phosphatase in heart. *Cell Signal* **18**, 1408-16.

Ito, M. (2004) Myosin phosphatase: structure, regulation and function. *Mol Cell Biochem* **259**, 197-209.



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