

#2535 Store at -20°C

# Phospho-AMPK $\alpha$ (Thr172) (40H9) Rabbit mAb

- Small 100  $\mu$ l (10 western blots)
- Large 300  $\mu$ l (30 western blots)



**Orders** ■ 877-616-CELL (2355)  
orders@cellsignal.com

**Support** ■ 877-678-TECH (8324)  
info@cellsignal.com

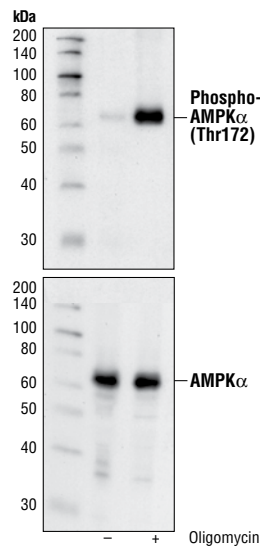
**Web** ■ www.cellsignal.com

rev. 08/12/11

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P Endogenous	H, M, R, Mk, Sc, Dm, Hm (C, B, Pg)	62 kDa	Rabbit IgG**

**Background:** AMP-activated protein kinase (AMPK) is highly conserved from yeast to plants and animals and plays a key role in the regulation of energy homeostasis (1). AMPK is a heterotrimeric complex composed of a catalytic  $\alpha$  subunit and regulatory  $\beta$  and  $\gamma$  subunits, each of which is encoded by two or three distinct genes ( $\alpha$ 1, 2;  $\beta$ 1, 2;  $\gamma$ 1, 2, 3) (2). The kinase is activated by an elevated AMP/ATP ratio due to cellular and environmental stress, such as heat shock, hypoxia and ischemia (1). The tumor suppressor LKB1, in association with accessory proteins STRAD and MO25, phosphorylates AMPK $\alpha$  at Thr172 in the activation loop and this phosphorylation is required for AMPK activation (3–5). AMPK $\alpha$  is also phosphorylated at Thr258 and Ser485 (for  $\alpha$ 1; Ser491 for  $\alpha$ 2). The upstream kinase and the biological significance of these phosphorylation events have yet to be elucidated (6). The  $\beta$ 1 subunit is post-translationally modified by myristoylation and multi-site phosphorylation including Ser24/25, Ser96, Ser101, Ser108 and Ser182 (6,7). Phosphorylation at Ser108 of the  $\beta$ 1 subunit seems to be required for the activation of AMPK enzyme, while phosphorylation at Ser24/25 and Ser182 affects AMPK localization (7). Several mutations in AMPK $\gamma$  subunits have been identified, most of which are located in the putative AMP/ATP binding sites (CBS or Bateman domains). Mutations at these sites lead to reduction of AMPK activity and cause glycogen accumulation in heart or skeletal muscle (1,2). Accumulating evidence indicates that AMPK not only regulates the metabolism of fatty acids and glycogen, but also modulates protein synthesis and cell growth through EF2 and TSC2/mTOR pathways, as well as blood flow via eNOS/nNOS (1).

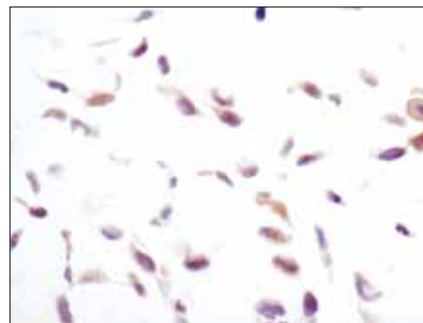


Western blot analysis of extracts from C2C12 cells, untreated or oligomycin-treated, using Phospho-AMPK $\alpha$  (Thr172) (40H9) Rabbit mAb (upper) or AMPK $\alpha$  Antibody #2532 (lower).

**Specificity/Sensitivity:** Phospho-AMPK $\alpha$  (Thr172) (40H9) Rabbit mAb detects endogenous AMPK $\alpha$  only when phosphorylated at threonine 172. The antibody detects both  $\alpha$ -1 and  $\alpha$ -2 isoforms of the catalytic subunit, but does not detect the regulatory  $\beta$  or  $\gamma$  subunits.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Thr172 of human AMPK $\alpha$  protein.

Immunohistochemical analysis of paraffin-embedded NCI-H228 cell pellets, control (left) or phenformin-treated (right), using Phospho-AMPK $\alpha$  (T172) (40H9) Rabbit mAb.



Entrez-Gene ID # 5562  
Swiss-Prot Acc. # Q13131

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

\*Species cross-reactivity is determined by western blot.

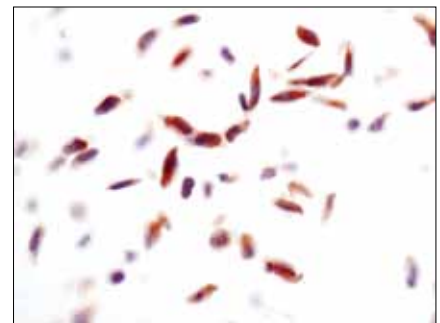
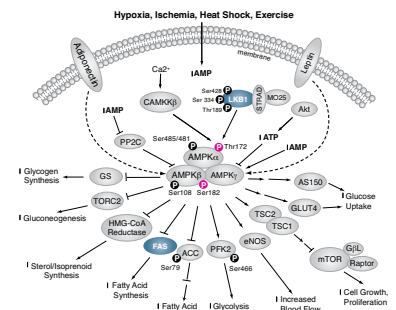
\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.

**Recommended Antibody Dilutions:**

- Western blotting 1:1000
- Immunoprecipitation 1:50
- Immunohistochemistry (Paraffin) 1:100†
- Unmasking buffer: Citrate
- Antibody diluent: SignalStain® Antibody Diluent #8112
- Detection reagent: SignalStain® Boost (HRP, Rabbit) #8114
- †Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.

For application specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.



**IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

Rabbit monoclonal antibody is produced under license (granting certain rights including those under U. S. Patents No. 5,675,063 and 7,429,487) from Epitomics, Inc.

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide

**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine

Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

**Background References:**

- (1) Hardie, D.G. (2004) *J. Cell Sci.* 117, 5479–5487.
- (2) Carling, D. (2004) *Trends Biochem. Sci.* 29, 18–24.
- (3) Hawley, S.A. et al. (1996) *J. Biol. Chem.* 271, 27879–27887.
- (4) Lizcano, J.M. et al. (2004) *EMBO J.* 23, 833–843.
- (5) Shaw, R. et al. (2004) *Proc. Natl. Acad. Sci. USA* 101, 3329–3335.
- (6) Woods, A. et al. (2003) *J. Biol. Chem.* 278, 28434–28442.
- (7) Warden, S.M. et al. (2001) *Biochem. J.* 354, 275–283.