

# Phospho-AMPK $\alpha$ (Thr172) Antibody

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

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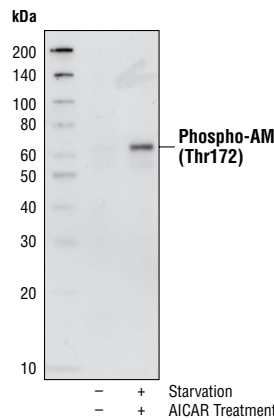
This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, M, R, Mk, (B, C, Pg)	62 kDa	Rabbit**

**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).

**Specificity/Sensitivity:** Phospho-AMPK $\alpha$  (Thr172) Antibody 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 it does not detect the regulatory  $\beta$  or  $\gamma$  subunits.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr172 of human AMPK $\alpha$ . Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of untreated and AICAR-treated C2C12 cell extracts using Phospho-AMPK $\alpha$  (Thr172) Antibody.

**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.

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 and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

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

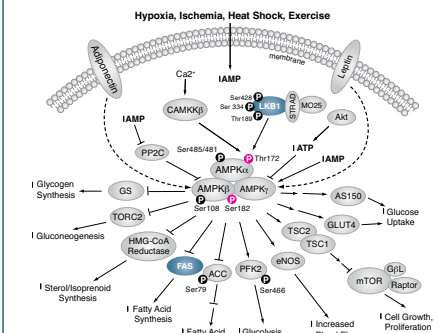
\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.

**Recommended Antibody Dilutions:**

Western blotting 1:1000

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% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

**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.