#9158 Store at –20°C

AMPK Control Cell Extracts

Controls for 10 western blots

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For Research Use Only. Not For Use In Diagnostic Procedures.

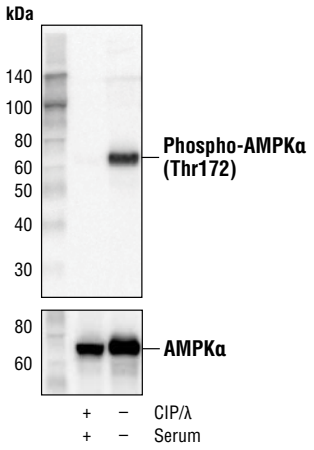
Product Includes	Product #	Quantity
AMPK Control Cell Extracts (C2C12 + CIP/λ)	78803	200 ul
AMPK Control Cell Extracts (C2C12 serum starved)	98827	200 ul

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 α subunit and regulatory β and γ subunits, each of which is encoded by two or three distinct genes (α1, 2; β1, 2; γ1, 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 AMPKα at Thr172 in the activation loop and this phosphorylation is required for AMPK activation (3-5). AMPKα is also phosphorylated at Thr258 and Ser485 (for α1; Ser491 for α2). The upstream kinase and the biological significance of these phosphorylation events have yet to be elucidated (6). The β1 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 β1 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 AMPKγ 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).

Description: *Nonphosphorylated AMPK Control Cell Extracts:* Total cell extracts from C2C12 cells, prepared with CIP/λ phosphatase, serve as a negative control. Supplied in SDS Sample Buffer.

Phosphorylated AMPK Control Cell Extracts: Total cell extracts from C2C12 cells, prepared by serum starvation, serve as a positive control. Supplied in SDS Sample Buffer.

Directions for Use: Boil for 3 minutes prior to use. Load 20 µl of nonphosphorylated and phosphorylated AMPK Control Cell Extract per lane.



Western blot analysis of extracts from C2C12 cells, serum starved or CIP/lambda treated prior to harvest, using Phospho-AMPKa (Thr172) (40H9) Rabbit mAb #2535 (upper) and AMPKa Antibody #5831 (lower).

Storage: *Supplied in SDS Sample Buffer:* 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red. Store at -20°C, or at -80°C for long-term storage.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

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.

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.