

#9957 Store at -20°C

AMPK and ACC Antibody Sampler Kit

✓ 1 Kit
(6 x 40 µl)



Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
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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.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-AMPK α (Thr172) (40H9) Rabbit mAb	2535	40 µl	62 kDa	Rabbit IgG
AMPK α (23A3) Rabbit mAb	2603	40 µl	62 kDa	Rabbit IgG
Phospho-AMPK β 1 (Ser108) Antibody	4181	40 µl	38 kDa	Rabbit IgG
AMPK β 1/2 (57C12) Rabbit mAb	4150	40 µl	34, 38 kDa	Rabbit IgG
Phospho-Acetyl-CoA Carboxylase (Ser79) Antibody	3661	40 µl	280 kDa	Rabbit IgG
Acetyl-CoA Carboxylase (C83B10) Rabbit mAb	3676	40 µl	280 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The AMPK and ACC Antibody Sampler Kit provides an economical means to investigate energy homeostasis and fatty acid synthesis within the cell. The kit contains primary and secondary antibodies to perform four Western blots with each antibody.

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 (α 1, 2; β 1, 2; γ 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 α at Thr172 in the activation loop and this phosphorylation is required for AMPK activation (3-5). AMPK α is also phosphorylated at Thr258 and Ser485 (for α 1; Ser491 for α 2). The upstream kinase and biological significance of these phosphorylation events have yet to be elucidated (6). The β 1 subunit is post-translationally modified by myristoylation and multi-site phosphorylation including Ser24/25, Ser96, Ser101 and Ser182 (6,7). Phosphorylation at Ser108 of the β 1 subunit seems to be required for the activation of AMPK enzyme, while phosphorylation at Ser24/25 and Ser182 affects AMPK localization (7). 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).

Acetyl-CoA carboxylase (ACC) catalyzes the pivotal step of the fatty acid synthesis pathway. The 265 kDa ACC α is the predominant isoform found in liver, adipocytes and mammary gland, while the 280 kDa ACC β is the major isoform in skeletal muscle and heart (8). Phosphorylation by AMPK at Ser79 or by PKA at Ser1200 inhibits the enzymatic activity of ACC (9). ACC is a potential target of anti-obesity drugs (10,11).

Specificity/Sensitivity: Phospho-AMPK α (Thr172) (40H9) Rabbit mAb detects endogenous AMPK α only when phosphorylated at Thr172. The antibody detects both α 1 and α 2 isoforms of the catalytic subunit, but does not detect the regulatory β or γ subunits. AMPK α (23A3) Rabbit mAb detects endogenous levels of total AMPK α protein. Phospho-AMPK β 1 (Ser108) Antibody detects endogenous levels of AMPK β 1 only when phosphorylated at Ser108. The antibody may cross-react with phosphorylated AMPK β 2 when phosphorylated at Ser109. AMPK β 1/2 (57C12) Rabbit mAb detects endogenous levels of both total AMPK β 1 and β 2 proteins. The antibody does not cross-react with other related proteins. Phospho-Acetyl-CoA Carboxylase (Ser79) Antibody detects endogenous levels of ACC only when phosphorylated at Ser79. The antibody recognizes both ACC α and ACC β . Acetyl-CoA Carboxylase (C83B10) Rabbit mAb detects endogenous levels of all isoforms of acetyl-CoA carboxylase protein.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser79 of rat Acetyl-CoA Carboxylase and Ser491 of human AMPK α 2. Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr172 of human AMPK α , the amino-terminal sequence of human AMPK α , residues surrounding His231 of AMPK β 1 and residues surrounding Ser52 of human Acetyl-CoA Carboxylase α 1. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

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

Recommended Antibody Dilutions:
Western blotting 1:1000

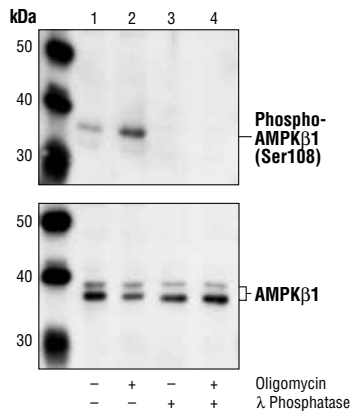
Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

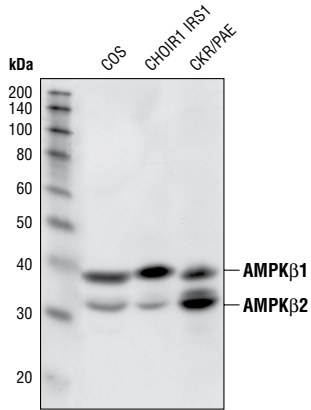
- (1) Hardie, D.G. (2004) *J. Cell Sci.* 117, 5479-5487.
- (2) Carling, D. (2004) *Trends Biochem. Sci.* 29, 18-24.
- (3) Hawley, S.A. et al. (1996) *J. Biol. Chem.* 271, 27879-27887.
- (4) Lizcano, J.M. et al. (2004) *EMBO J.* 23, 833-843.
- (5) Shaw, R.J. et al. (2004) *Proc. Natl. Acad. Sci. U S A* 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.
- (8) Ruderman, N.B. et al. (1999) *Am. J. Physiol.* 276, E1-E18.
- (9) Ha, J. et al. (1994) *J. Biol. Chem.* 269, 22162-22168.
- (10) Abu-Elheiga, L. et al. (2001) *Science* 291, 2613-2616.
- (11) Levert, K.L. et al. (2002) *J. Biol. Chem.* 277, 16347-16350.

Selected rabbit monoclonal antibodies are produced under license (granting certain rights including those under U.S. Patent No. 5,675,063 and/or U.S.S.N. 11/476,277) from Epitomics, Inc. U.S.S.N. 11/476,277) from Epitomics, Inc.

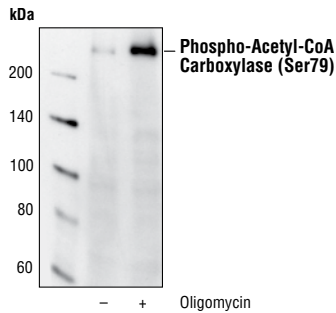
Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence F—Flow cytometry E—ELISA D—DELFI[®]
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine All—all species expected
Species enclosed in parentheses are predicted to react based on 100% sequence homology.



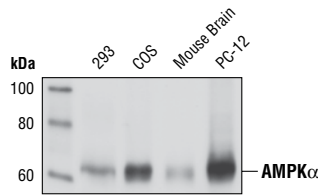
Western blot analysis of extracts from C2C12 cells, untreated (lanes 1,3) or oligomycin-treated (lanes 2,4), using **Phospho-AMPKβ1 (Ser108) Antibody #4181** (upper) or **AMPKβ1 Antibody #4182** (lower). Cell lysates were treated with λ phosphatase in lanes 3 and 4 to demonstrate the phospho-specificity of Phospho-AMPKβ1 (Ser108) Antibody.



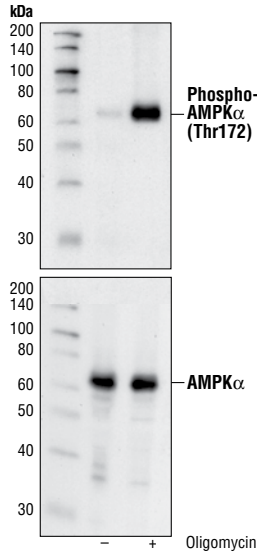
Western blot analysis of cell lysates from various cell types using **AMPKβ1/2 (57C12) Rabbit mAb #4150**.



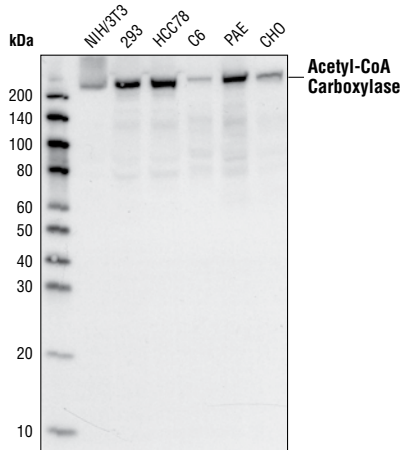
Western blot analysis of extracts from HEK293 cells, untreated or oligomycin-treated, using **Phospho-Acetyl-CoA Carboxylase (Ser79) Antibody #3661**.



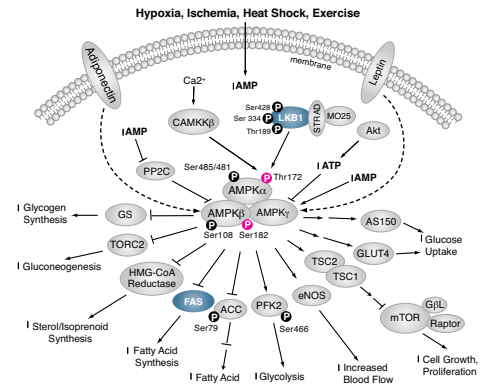
Western blot analysis of extracts from various cells and tissues using **AMPKα (23A3) Rabbit mAb #2603**.



Western blot analysis of extracts from C2C12 cells, untreated or oligomycin-treated (0.5 μM), using **Phospho-AMPKα (Thr172) (40H9) Rabbit mAb #2535** (upper) or **AMPKα Antibody #2532** (lower).



Western blot analysis of cell extracts from various cell types using **Acetyl-CoA Carboxylase (C83B10) Rabbit mAb #3676**.



Western Immunoblotting Protocol (Primary Ab Incubation In BSA)

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.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X 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
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.