Store at -20°C

#9957

AMPK and ACC Antibody Sampler Kit

☑ 1 Kit
(6 x 20 µl)

Cell Signaling TECHNOLOGY®

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

rev. 04/04/17

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-AMPKα (Thr172) (40H9) Rabbit mAb	2535	20 µl	62 kDa	Rabbit IgG
AMPKα (D5A2) Rabbit mAb	5831	20 µl	62 kDa	Rabbit IgG
Phospho-AMPKβ1 (Ser182) Antibody	4186	20 µl	38 kDa	Rabbit IgG
AMPKβ1/2 (57C12) Rabbit mAb	4150	20 µl	30, 38 kDa	Rabbit IgG
Phospho-Acetyl-CoA Carboxylase (Ser79) (D7D11) Rabbit mAb	11818	20 µl	280 kDa	Rabbit IgG
Acetyl-CoA Carboxylase (C83B10) Rabbit mAb	3676	20 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.

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.

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 includes enough antibody to perform two western blot experiments with each primary 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 (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 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 and Ser182 (6,7). Phosphorylation at Ser108 of the β1 subunit seems to be required for the activation of AMPK enzyme, while phosphorylation ot 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 ACCα is the predominant isoform found in liver, adipocytes and mammary gland, while the 280 kDa ACCβ 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-AMPKα (Thr172) (40H9) Rabbit mAb detects endogenous AMPKα only when phosphorylated at Thr172. The antibody detects both α1 and α2 isoforms of the catalytic subunit, but does not detect the regulatory β or γ subunits. AMPKα (D5A2) Rabbit mAb detects endogenous levels of total AMPKα protein. Phospho-AMPKβ1 (Ser182) Antibody detects endogenous levels of total AMPKβ1 only when phosphorylated at Ser182. AMPKβ1/2 (57C12) Rabbit mAb detects endogenous levels of both total AMPKβ1 and β2 proteins. The antibody does not cross-react with other related proteins. Phospho-Acetyl-CoA Carboxylase (Ser79) (D7D11) Rabbit mAb recognizes endogenous levels of acetyl-CoA carboxylase protein only when phosphorylated at Ser79. Acetyl-CoA Carboxylase (C83B10) Rabbit mAb detects endogenous levels of all isoforms of acetyl-CoA carboxylase protein.

Source/Purification:

Monoclonal state-specific antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr172 of human AMPKα, and Ser79 of human ACC. Total monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Arg21 of human AMPKα, His233 of human AMPKß1, and Ser523 of human Acetyl-CoA Carboxylase α1. Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser182 of human AMPKß1. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

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. 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.
(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.

U.S. Patent No. 5,675,063

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **IC**—Immunocytochemistry **IF**—Immunofluorescence **F**—Flow cytometry **E**—ELISA **D**—DELFIA®

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 Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.