AMPK and ACC Antibody Sampler Kit

1 Kit
(6 x 20 µl)

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 homostasis 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 homostasis (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 AMPKα1 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 of 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).

Species Cross-Reactivity: Species cross-reactivity is expected.

Source/ Purification:
Monoclonal state-specific antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr172 of human AMPKα1 and Ser79 of human ACC. Total monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Arg21 of human AMPKα1, His233 of human AMPKβ1, and Ser523 of human Acetyl-CoA Carboxylase α1. Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr172 of human AMPKα1.

See www.cellsignal.com for a complete listing of recommended companion products.
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 dH₂O, mix.
2. 10X Tris Buffered Saline (TBS): (#12496) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH₂O, 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 3X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH₂O.
4. 10X Tris-Glycine SDS Running Buffer: (#4050) To prepare 1 L 1X running buffer: add 100 ml 1X running buffer to 900 ml dH₂O, mix.
5. 10X Tris-Glycine Transfer Buffer: (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X Tris-glycine transfer buffer to 900 ml dH₂O, mix.
6. 10X Tris Buffered Saline with Tween® 20 (TBST): (#9997) To prepare 1 L 1X TBST: add 100 ml 1X TBST to 900 ml dH₂O, 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.
13. Prestained Protein Marker, Broad Range (Premixed Format): (#7720)
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 (do 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) to determine molecular weights are recommended.
8. Electrophoresis to nitrocellulose membrane (#12369).

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.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBST 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.