

AMPK Subunit Antibody Sampler Kit

✓ 1 Kit
(7 x 40 µl)

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This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
AMPK α (23A3) Rabbit mAb	2603	40 µl	62 kDa	Rabbit IgG
AMPK α 2 Antibody	2757	40 µl	62 kDa	Rabbit IgG
AMPK β 1 (71C10) Rabbit mAb	4178	40 µl	38 kDa	Rabbit IgG
AMPK β 2 Antibody	4148	40 µl	30 kDa	Rabbit IgG
AMPK γ 1 Antibody	4187	40 µl	37 kDa	Rabbit IgG
AMPK γ 2 Antibody	2536	40 µl	75 kDa	Rabbit IgG
AMPK γ 3 Antibody	2550	40 µl	54 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

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

Description: The AMPK Subunit Antibody Sampler Kit provides an economical means to investigate the role played by all AMPK subunits in cellular energy homeostasis. The kit contains enough primary and secondary antibodies to perform four western mini-blot 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 the 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, Ser108 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). Several mutations in AMPK γ 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: Each of the antibodies in the AMPK Subunit Antibody Sampler Kit detects endogenous levels of the specified AMPK protein. Antibodies do not cross-react with related AMPK subunit proteins.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with synthetic peptides (KLH-coupled) corresponding to residues surrounding Ser500 of human AMPK α 2, near the amino terminus of human AMPK γ 1, surrounding Ser60 of human AMPK γ 2, and derived from the sequences of human AMPK β 2 and AMPK γ 3. Antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with synthetic peptides (KLH-coupled) derived from the amino-terminal sequence of human AMPK α and corresponding to residues surrounding Val176 of human AMPK β 1.

Background References:

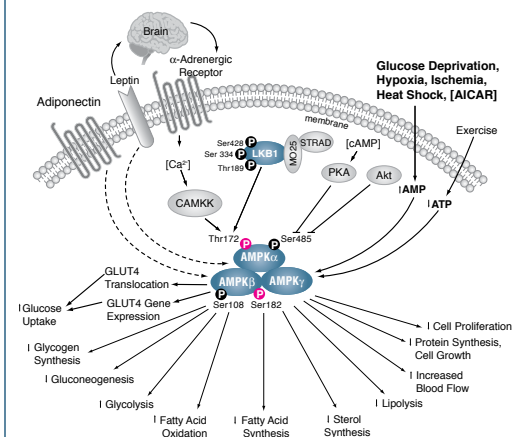
- (1) Hardie, D.G. (2004) *J Cell Sci* 117, 5479-87.
- (2) Carling, D. (2004) *Trends Biochem Sci* 29, 18-24.
- (3) Hawley, S.A. et al. (1996) *J Biol Chem* 271, 27879-87.
- (4) Lizcano, J.M. et al. (2004) *EMBO J* 23, 833-43.
- (5) Shaw, R.J. et al. (2004) *Proc Natl Acad Sci USA* 101, 3329-35.
- (6) Woods, A. et al. (2003) *J Biol Chem* 278, 28434-42.
- (7) Warden, S.M. et al. (2001) *Biochem J* 354, 275-83.

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

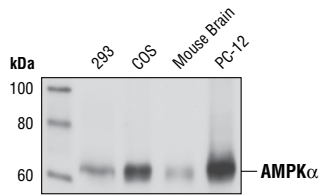
Please visit www.cellsignaling.com for a complete listing of recommended companion products.



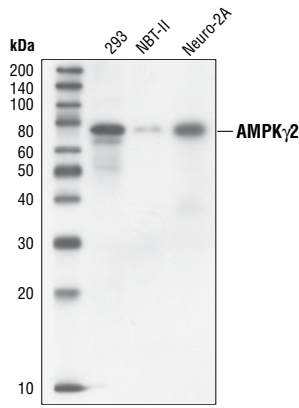
Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide

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

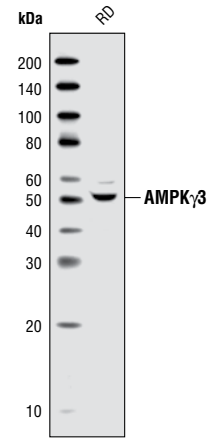
Dg—dog Pg—pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



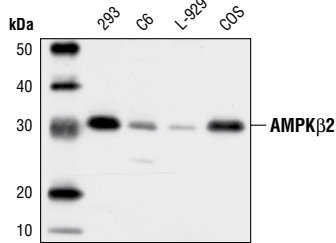
Western blot analysis of extracts from 293 and COS cells, mouse brain and PC-12 cells using **AMPK α (23A3) Rabbit mAb #2603**.



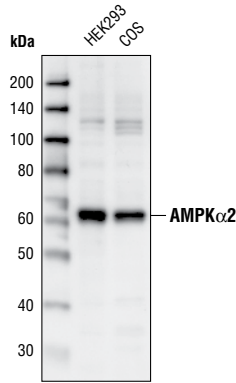
Western blot analysis of extracts from 293 (human), NBT-II (rat), and Neuro-2A (mouse) cells using **AMPK γ 2 Antibody #2536**.



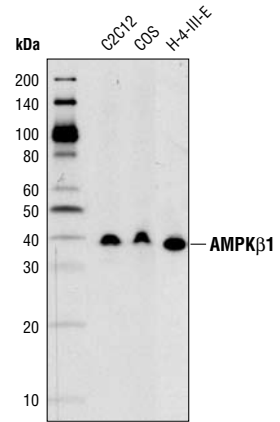
Western blot analysis of extracts from RD cells using **AMPK γ 3 Antibody #2550**.



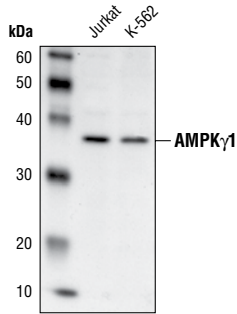
Western blot analysis of extracts from 293, C6, L-929 and COS cells using **AMPK β 2 Antibody #4148**.



Western blot analysis of extracts from HEK293 and COS cells using **AMPK α 2 Antibody #2757**.



Western blot analysis of extracts from C2C12, COS and H-4-III-E cells using **AMPK β 1 (71C10) Rabbit mAb #4178**.



Western blot analysis of extracts from Jurkat and K-562 cells using **AMPK γ 1 Antibody #4187**.

Western Immunoblotting Protocol (Primary Antibody 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.