Rabbit (polyclonal) Anti-AMPKα 1/2 [pT^{172}] Phosphospecific Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number:	44-1150G (10 mini-blot size)
Lot Number:	See product label
Volume:	100 µL
Form of Antibody:	Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier.
Preservative:	0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)
Purification:	Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated AMPK. The final product is generated by affinity chromatography using a AMPK-derived peptide that is phosphorylated at threonine 172.
Immunogen:	The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of human AMPK that contains threonine 172. The sequence is conserved in human, mouse and rat.
Target Summary:	AMP-activated protein kinase (AMPK) is a metabolic and stress-sensing kinase that regulates homeostatis, and a key target for treating Type 2 diabetes and obesity. AMPK exists as a heterotrimeric complex comprised of catalytic alpha subunits (62 kDa) and non-catalytic beta and gamma subunits. Alpha subunit has at least two isoforms (alpha 1 and alpha 2) which differ in their subcellular localization and AMP-dependence. AMPK is phosphorylated by upstream kinases, AMPK Kinase (AMPKK) and LKB1 which results in its activation. Active AMPK regulates metabolism by phosphorylating rate-limiting enzymes in metabolic pathways and controlling gene expression. Phosphorylation of threonine 172 in the activation loop of the alpha subunit is a key determinant of AMPK activity.
Cross-Reactivity:	Human, mouse and rat.
Applications:	The antibody has been used in Western blotting. Other applications have not been tested at Invitrogen.
Suggested Working Dilutions:	For Western blotting applications, we recommend using the antibody at a 1:1000 starting dilution. The optimal antibody concentration should be determined empirically for each specific application.
Storage:	Store at –20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at –20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.
Expiration Date:	Expires one year from date of receipt when stored as instructed.
Positive Controls Used:	Insulin-treated CHO-T cells, Insulin-treated 3T3L1, Metformin-treated L6 myoblast cells, Metformin-treated HepG2 cells

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Related Products:

Antibodies:

IR/IGFR [pY^{1158}], Cat. # 44-802G

Antibodies (continued):

IRS-1 [pY^{612}], Cat. # 44-816G

AS160 [pT^{642}], Cat. # 44-1071G

mTOR [pT^{2448}], Cat. # 44-1125G

GSK3β [pS^{9}], Cat. # 44-600G

Akt [pS^{473}], Cat. # 44-623G

PTEN [pSpTpS$^{380/382/385}$], Cat. #44-1066G

Akt [pT^{308}], Cat. # 44-602G

P70 S6 Kinase [pT^{229}], Cat. # 44-918G

RSK1 [pS^{221}], Cat. # 44-924G

RSK1 [pS^{363}], Cat. # 44-926G

References:

McCullough, L.D. et al. (2005) Pharmacological inhibition of AMP-activated protein kinase provides neuroprotection in stroke. J. Biol. Chem. 280(21):20493-20502.

Jorgensen, S.B. et al. (2004) The alpha2-5'AMP-activated protein kinase is a site 2 glycogen synthase kinase in skeletal muscle and is responsive to glucose loading. Diabetes 53(12):3074-3081.

Shaw, R.J. et al. (2004) The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. Proc. Natl. Acad. Sci. USA. 101(10):3329-3335.

Zhou, M.H. et al. (2004) Activation of the AMP-activated protein kinase by the anti-diabetic drug metformin in vivo. Role of mitochondrial reactive nitrogen species. J. Biol. Chem. 279(42):43940-43951.

Woods, A. et al. (2003) Identification of phosphorylation sites in AMP-activated protein kinase (AMPK) for upstream AMPK kinases and study of their roles by site-directed mutagenesis. J. Biol. Chem. 2003 Aug 1;278(31):28434-28442.

Yin, W. et al. (2003) Role of AMP-activated protein kinase in cyclic AMP-dependent lipolysis in 3T3-L1 adipocytes. J. Biol. Chem. 2003 Oct 31;278(44):43074-43080.

Nagata, et al. (2003) AMP-activated protein kinase (AMPK) signaling in endothelial cells is essential for angiogenesis in response to hypoxic stress. J. Biol. Chem. 278(33):31000-31006.

Hawley, S.A. et al. (2002) The antidiabetic drug metformin activates the AMP-activated protein kinase cascade via an adenine nucleotide-independent mechanism. Diabetes 51(8):2420-2425.

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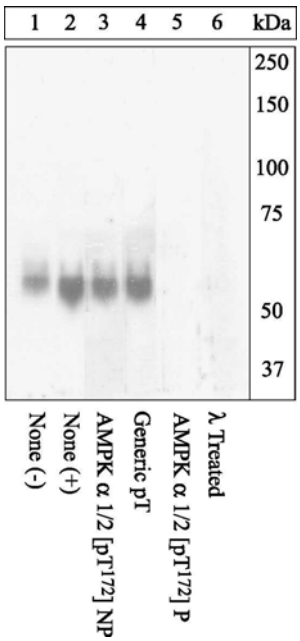

Upregulation, Antibody-Peptide Competition and Phosphatase Stripping

Extracts of HepG2 cells untreated (1) or treated with 12 mM Metformin for 24 hours in serum free media (2-6) were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. The membrane was left untreated (1-5) or treated with lambda phosphatase (6), blocked with a 3% BSA-TBST buffer for one hour at room temperature, and then incubated with the AMPKα 1/2 [pT ^{172}] antibody for two hours at room temperature in 3% BSA-TBST buffer, following prior incubation with: no peptide (1,2), the non-phosphopeptide corresponding to the phosphopeptide immunogen (3), a generic phosphothreonine-containing peptide (4), or the phosphopeptide immunogen (5). After washing, the membrane was incubated with goat F(ab')$_2$ anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and signals were detected using the Pierce SuperSignal™ method.

The data show that only the phosphopeptide corresponding to AMPKα 1/2 [pT^{172}] completely blocks the signal and that phosphatase stripping eliminates the signal, verifying that the antibody is phosphorylation site-specific. The data also show upregulation of the phospho-signal upon Metformin treatment in this cell system.

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Western Blotting Procedure

1. Lyse approximately 10^7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Invitrogen International catalog number FNN0011). Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH2O for 5 minutes.
7. Soak the PVDF membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) overnight at 4°C or for one hour at room temperature.
12. Incubate the blocked blot with primary antibody at a 1:1000 starting concentration in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween 20 overnight at 4°C or for two hours at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase conjugate (catalog number ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (catalog number ALI4404) in conjunction with your chemiluminescence reagents and instrumentation.

Cell Lysis Buffer Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM Na_3VO_4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO) or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma Cat. # P2714 may be used)

Transfer Buffer Formulation:
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add
1 mL 10% SDS.
Cool to 4°C prior to use.

Tris Buffered Saline Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer Formulation:
100 mL Tris buffered saline
3 gm Ig-free BSA
0.1 mL Tween 20

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