Anti-phospho-AMPKα (Thr172)

Polyclonal Antibody

Cat. # 07-626

Lot # 31993

pack size: 200 µL

Store at -20°C

FOR RESEARCH USE ONLY

Certificate of Analysis

Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/ Region	Host Species	Molecular Weight	Accession #
WB	H	IgG	N/A	Rb	~63 kDa	NP_006242

Background

AMP-activated protein kinase (AMPK) is a metabolic and stress-sensing kinase that regulates homeostasis, and a key target for treating Type 2 diabetes and obesity. AMPK is an important regulator of glucose uptake, oxidation of fatty acids, cholesterol synthesis, lipogenesis, triglyceride synthesis, adipocyte lipolysis, glycolysis, and nitric oxide synthesis. AMPK is activated by exercise and more specifically, muscle contraction. During exercise, AMPK inhibits the enzyme acetyl-CoA carboxylase by phosphorylating the Ser79 site. AMPK exists as a heterotrimeric complex comprised of catalytic alpha subunits 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.

Presentation

Purified rabbit polyclonal IgG in buffer containing 70% storage buffer 0.2 M Tris-glycine, pH 7.4, 0.15 M NaCl, 5 mg/mL BSA and 0.05% sodium azide and 30% glycerol.

Specificity

Recognizes phospho-AMPKα (Thr172), MW ~63 kDa. A non-specific protein was also detected, MW ~160 kDa.

Species Cross-reactivity

Human. Predicted to cross-react with rat based on sequence similarity.

Immunogen

KLH-conjugated, synthetic peptide corresponding to amino acids surrounding phosphorylated Thr172 of human AMP-activated protein kinase alpha 2 (AMPKα2). The immunogenic site is conserved in AMPKα1, isoform 1 (Thr174), AMPKα1, isoform 2 (Thr189), and AMPKα2 (Thr172). Accession number NM_006251. The immunizing sequence is identical in rat.

Molecular Weight

~63 kDa

Method of Purification

Immunoaffinity Purified

Storage and Handling

Stable for 1 year at -20°C from date of receipt.

Handling Recommendations: Upon first thaw, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance. Note: Variabillity in freezer temperatures below -20°C may cause glycerol containing solutions to become frozen during storage.

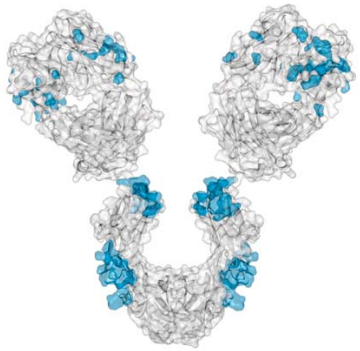
Control

Serum starved HEK293 cells

Quality Control Testing

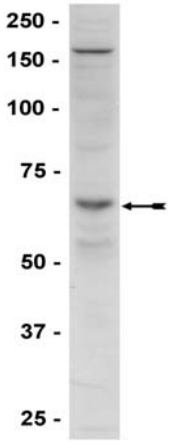
Evaluated by western blot in RIPA lysates from serum starved HEK293 cells.

Western Blot Analysis: 1:500-1:2000 dilutions of this antibody detected phospho-AMPKα (Thr172) in RIPA lysates from serum starved HEK293 cells.



References

1. Kemp, B.E., *et al.* (1999). *TIBS.* 24: 22-25.
2. Michell, B.J., *et al.* (1996). *J. Biol. Chem*. 271: 28445-28450.



Western Blot Analysis
Representative lot data.
RIPA lysate from serum starved HEK293 cells was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-AMPKα (Thr172) (1:1000 dilution).
Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system.

Arrow indicates phospho-AMPKα (Thr172) (~63 kDa).

PROTOCOL

Western Blot

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 µg/mL each aprotinin, leupeptin, pepstatin; 1 mM Na3VO4, 1 mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared TBS containing 5% nonfat dry milk (Catalog # 20-200) and 0.05% Tween®-20 (TBST-MLK) for 30 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with a **1:500-1:2000 dilution of anti-phospho-AMPKα (Thr172)**, diluted in freshly prepared TBST-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in TBST-MLK for 1.5 hours with agitation at room temperature.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-0.05% Tween®-20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

RELATED PRODUCTS (specific)

cat #	description
07-181	Anti-AMPK α-pan
07-626	Anti-phospho-AMPK α (Thr172)
07-681	Anti-phospho-AMPK α (Thr172)
12-480	AMPK α-pan Immunizing Peptide
14-305	AMPK, active
12-355	SAMS Peptide
32-007	AMPK KinEASE™ FP Fluorescein Green Assay
32-087	AMPK KinEASE™ FP-645nm FarRed Assay
07-350	Anti-AMPK α1
04-323	Anti-AMPKα1, Rabbit Monoclonal
M-005027	AMPK α1 SMARTpool® siRNA reagent
60-101	AMPK α1 siRNA/siAb™ Assay Kit
62-147	siRNA plasmid, pKD-AMPKα1-v1
07-363	Anti-AMPK α2
M-005361	AMPK α2 SMARTpool® siRNA reagent
60-108	AMPK α2 siRNA/siAb™ Assay Kit
07-670	Anti-AMPK β
04-324	Anti-AMPKβ1, Rabbit Monoclonal

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPFL00010	Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPVH07850	Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	Immobilon Western Chemilum HRP Substrate 100 mL
17-373	Spray & Glow™ ECL WB Detection System 1 ea
2060	Re-Blot Western Blot Recycling Kit
2500	Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	Blot Quick Blocker Membrane Blocking Agent 175G