abcam

Product datasheet

Anti-AMPK alpha 1 antibody [Y365] ab32047

KO VALIDATED Recombinant RabMAb

★★★★★ 6 Abreviews 165 References 12 Images

Overview

Product name	Anti-AMPK alpha 1 antibody [Y365]
Description	Rabbit monoclonal [Y365] to AMPK alpha 1
Host species	Rabbit
Specificity	This antibody is specific for human AMPK alpha 1. This antibody shows low affinity on mouse and rat samples.
Tested applications	**Suitable for:** ICC/IF, Flow Cyt (Intra), WB, IHC-P, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** African green monkey
Immunogen	Synthetic peptide within Human AMPK alpha 1 aa 500 to the C-terminus (C terminal). The exact sequence is proprietary. Database link: **Q13131**
Positive control	WB: HeLa, HepG2, C6, NIH/3T3 and MCF-7 cell lysate. Mouse liver, brain, retina, and skeletal muscle tissue lysates, RAW 264.7 cell lysate, Neuro2A cell lysate. IHC-P: Human cervical carcinoma and lung carcinoma tissues. ICC/IF: MCF-7 cells. Flow Cyt (intra): HeLa cells. IP: HeLa whole cell lysate (**ab150035**).
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20

	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y365
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32047 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

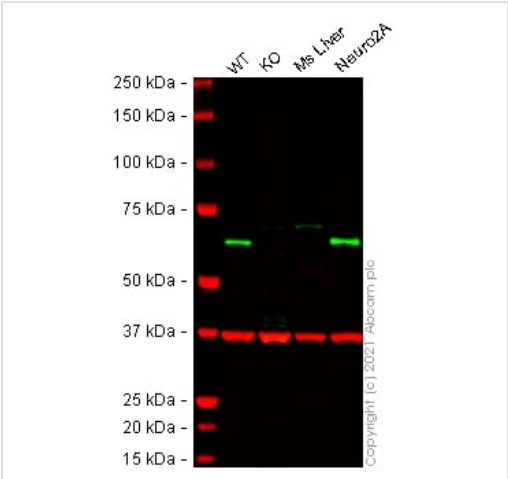
Application	Abreviews	Notes
ICC/IF		1/250.
Flow Cyt (Intra)		1/100 - 1/150. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ **(5)**	1/1000 - 1/5000. Predicted molecular weight: 63 kDa. This antibody shows low affinity on mouse and rat samples.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. Mouse and rat species are recommended by WB application.
IP		1/40 - 1/50.

Target

Function Responsible for the regulation of fatty acid synthesis by phosphorylation of acetyl-CoA carboxylase. It also regulates cholesterol synthesis via phosphorylation and inactivation of hormone-sensitive lipase and hydroxymethylglutaryl-CoA reductase. Appears to act as a metabolic stress-sensing protein kinase switching off biosynthetic pathways when cellular ATP levels are depleted and when 5'-AMP rises in response to fuel limitation and/or hypoxia. This is a catalytic subunit.

Sequence similarities Belongs to the protein kinase superfamily. CAMK Ser/Thr protein kinase family. SNF1 subfamily. Contains 1 protein kinase domain.

Images



Western blot - Anti-AMPK alpha 1 antibody [Y365] (ab32047)

All lanes : Anti-AMPK alpha 1 antibody [Y365] (ab32047) at 1/1000 dilution

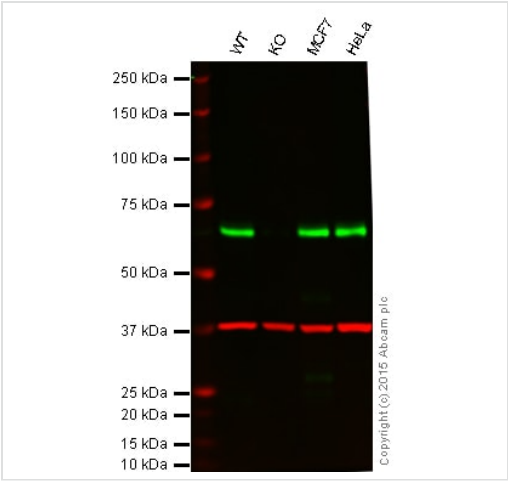
Lane 1 : Wild-type RAW 264.7 cell lysate
Lane 2 : PRKAA1 knockout RAW 264.7 cell lysate
Lane 3 : Mouse Liver cell lysate
Lane 4 : Neuro2A cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 63 kDa
Observed band size: 64 kDa

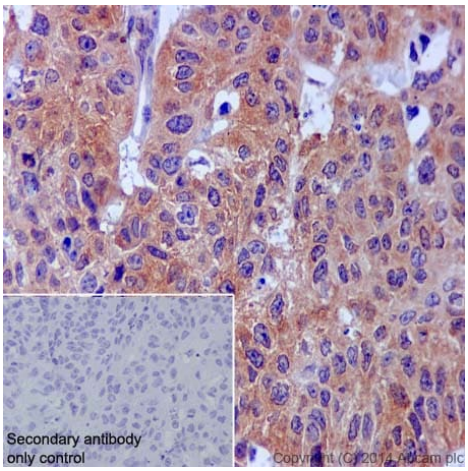
False colour image of Western blot: Anti-AMPK alpha 1 antibody [Y365] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32047 was shown to bind specifically to AMPK alpha 1. A band was observed at 64 kDa in wild-type RAW 264.7 cell lysates with no signal observed at this size in PRKAA1 knockout cell line **ab280055** (knockout cell lysate **ab280114**). To generate this image, wild-type and PRKAA1 knockout RAW 264.7 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged.Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-AMPK alpha 1 antibody [Y365] (ab32047)

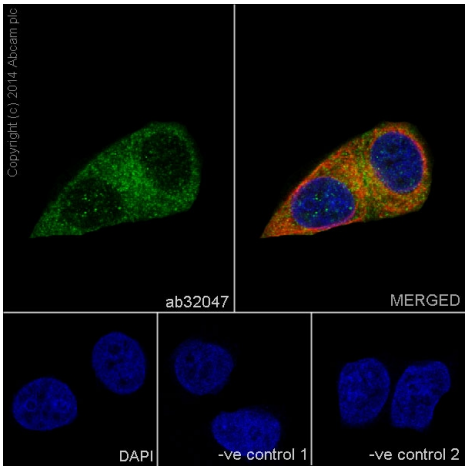
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: AMPK alpha knockout HAP1 cell lysate (20 µg)
Lane 3: MCF7 cell lysate (20 µg)
Lane 4: HeLa cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab32047 observed at 63 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab32047 was shown to specifically react with AMPK alpha in wild-type HAP1 cells. No band was observed when AMPK alpha knockout samples were examined. Wild-type and AMPK alpha knockout samples were subjected to SDS-PAGE. ab32047 and **ab8245** (loading control to GAPDH) were diluted 1/5000 and 1/2000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10,000 dilution for 1hr at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AMPK alpha 1 antibody [Y365] (ab32047)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human lung carcinoma tissue labelling AMPK alpha 1 with purified ab32047 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9 before commencing with IHC staining protocol. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

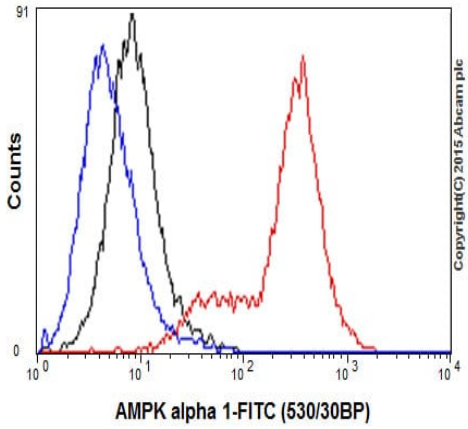


Immunocytochemistry/ Immunofluorescence - Anti-AMPK alpha 1 antibody [Y365] (ab32047)

Immunocytochemistry/Immunofluorescence analysis of MCF7 cells labelling AMPK alpha 1 with purified ab32047 at 1/250. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500) were also used.

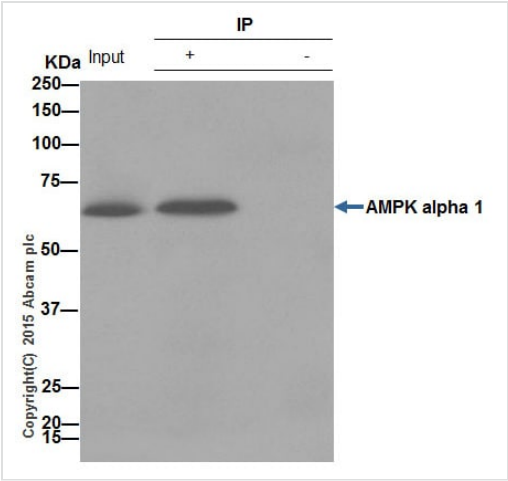
Control 1: primary antibody (1/250) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).



Flow Cytometry (Intracellular) - Anti-AMPK alpha 1 antibody [Y365] (ab32047)

Intracellular Flow Cytometry analysis of HeLa cells labelling AMPK alpha 1 with purified ab32047 at 1/150 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Immunoprecipitation - Anti-AMPK alpha 1 antibody [Y365] (ab32047)

ab32047 (purified) at 1/40 immunoprecipitating AMPK alpha 1 in HeLa whole cell lysate.

Lane 1 (input): HeLa whole cell lysate (10µg)

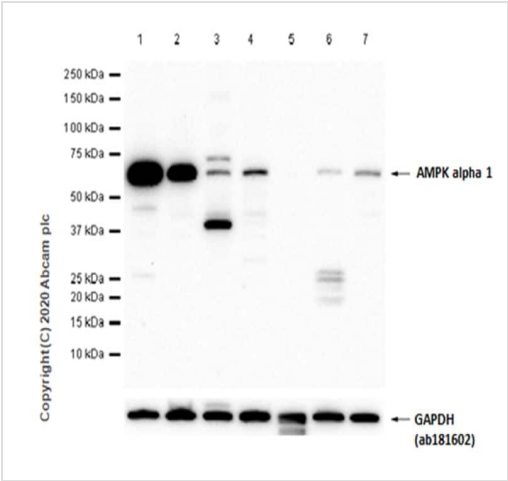
Lane 2 (+): ab32047 + HeLa whole cell lysate (10µg).

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32047 in HeLa whole cell lysate.

For western blotting, **ab131366** VeriBlot for IP Detection Reagent (HRP) was used for detection (1/1500).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-AMPK alpha 1 antibody [Y365] (ab32047)

All lanes : Anti-AMPK alpha 1 antibody [Y365] (ab32047) at 1/500 dilution

Lane 1 : HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate
Lane 3 : Mouse liver tissue lysate
Lane 4 : Mouse brain tissue lysate
Lane 5 : Mouse kidney tissue lysate
Lane 6 : Mouse retina tissue lysate
Lane 7 : Mouse skeletal muscle tissue lysate

Lysates/proteins at 20 µg per lane.

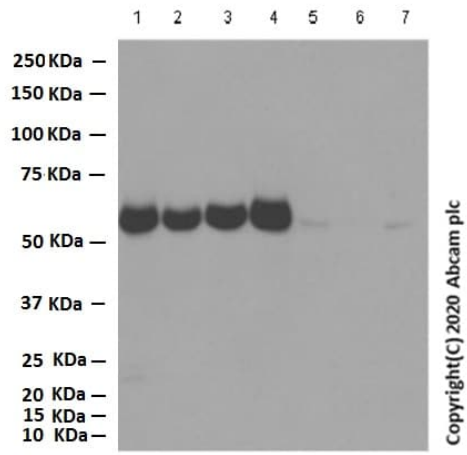
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 63 kDa
Observed band size: 63 kDa
Additional bands at: 40 kDa (possible non-specific binding)

Exposure time: 3 minutes

Blocking/Diluting buffer and concentration: 5% NFDM/TBST.

This antibody shows low affinity on mouse samples.



Western blot - Anti-AMPK alpha 1 antibody [Y365] (ab32047)

All lanes : Anti-AMPK alpha 1 antibody [Y365] (ab32047) at 1/20000 dilution

Lane 1 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysate
Lane 3 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 4 : K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysate
Lane 5 : C6 (Rat glial tumor glial cell) whole cell lysate
Lane 6 : Neuro-2a (Mouse neuroblastoma neuroblast) whole cell lysate
Lane 7 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

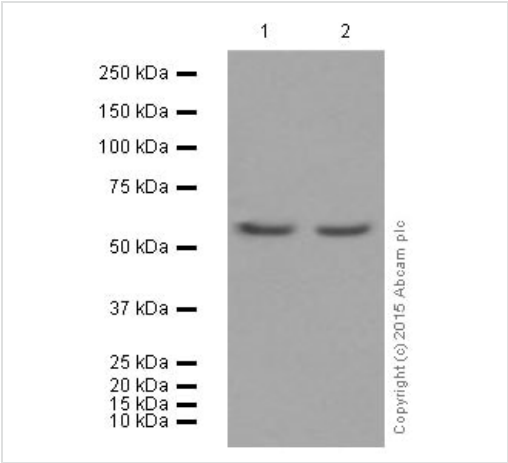
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 63 kDa
Observed band size: 63 kDa

Exposure time: 180 seconds

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

This antibody shows low affinity on mouse and rat samples.



Western blot - Anti-AMPK alpha 1 antibody [Y365] (ab32047)

All lanes : Anti-AMPK alpha 1 antibody [Y365] (ab32047) at 1/2000 dilution (purified)

Lane 1 : C6 cell lysate

Lane 2 : NIH/3T3 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

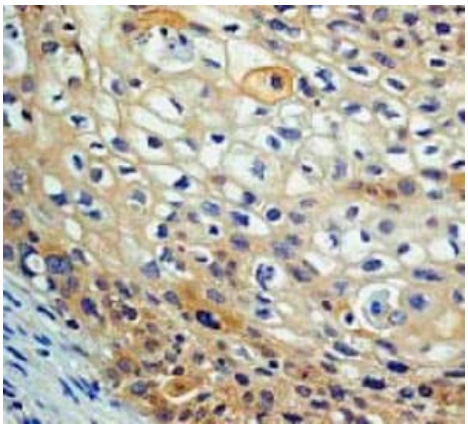
All lanes : Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

Predicted band size: 63 kDa

Observed band size: 63 kDa

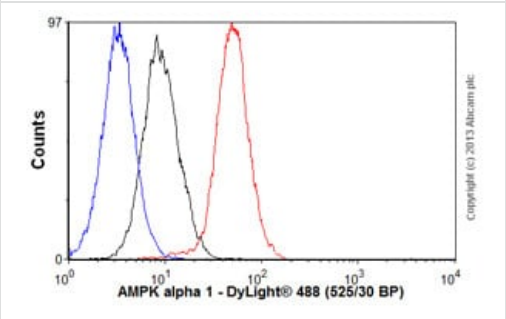
Blocking and dilution buffer: 5% NFDM/TBST.

This antibody shows low affinity on mouse and rat samples.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AMPK alpha 1 antibody [Y365] (ab32047)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analaysis of human cervical carcinoma tissue labelling AMPK alpha 1 with unpurified ab32047 at a dilution of 1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-AMPK alpha 1 antibody [Y365] (ab32047)

Overlay histogram showing HeLa cells stained with unpurified ab32047 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (unpurified ab32047, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

Anti-AMPK alpha 1 antibody [Y365] (ab32047)

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors