abcam

Product datasheet

Anti-AMPK gamma 1 antibody [Y307] - BSA and Azide free ab247251





RabMAb

4 Images

Overview

Product name Anti-AMPK gamma 1 antibody [Y307] - BSA and Azide free

Description Rabbit monoclonal [Y307] to AMPK gamma 1 - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: IHC-P, WB

Species reactivity Reacts with: Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Epitope ab247251 reacts with an epitope located in the N terminal region of AMPK gamma 1.

General notes ab247251 is the carrier-free version of <u>ab32382</u>.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar $^{\circledR}$ Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar $^{\circledR}$ is a trademark of Fluidigm Canada Inc.

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**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal

Clone number Y307 Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab247251 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
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Predicted molecular weight: 38 kDa.

Target

Function

AMP/ATP-binding subunit of AMP-activated protein kinase (AMPK), an energy sensor protein kinase that plays a key role in regulating cellular energy metabolism. In response to reduction of intracellular ATP levels, AMPK activates energy-producing pathways and inhibits energy-consuming processes: inhibits protein, carbohydrate and lipid biosynthesis, as well as cell growth and proliferation. AMPK acts via direct phosphorylation of metabolic enzymes, and by longer-term effects via phosphorylation of transcription regulators. Also acts as a regulator of cellular polarity by remodeling the actin cytoskeleton; probably by indirectly activating myosin. Gamma non-catalytic subunit mediates binding to AMP, ADP and ATP, leading to activate or inhibit AMPK: AMP-binding results in allosteric activation of alpha catalytic subunit (PRKAA1 or PRKAA2) both by inducing phosphorylation and preventing dephosphorylation of catalytic subunits. ADP also stimulates phosphorylation, without stimulating already phosphorylated catalytic subunit. ATP promotes dephosphorylation of catalytic subunit, rendering the AMPK enzyme inactive.

Sequence similarities

Belongs to the 5'-AMP-activated protein kinase gamma subunit family.

Contains 4 CBS domains.

Domain

The AMPK pseudosubstrate motif resembles the sequence around sites phosphorylated on

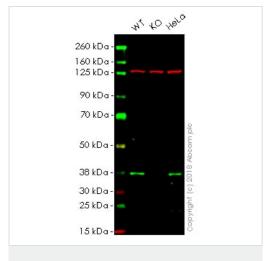
target proteins of AMPK, except the presence of a non-phosphorylatable residue in place of Ser. In the absence of AMP this pseudosubstrate sequence may bind to the active site groove on the alpha subunit (PRKAA1 or PRKAA2), preventing phosphorylation by the upstream activating kinase STK11/LKB1.

The CBS domains mediate binding to AMP, ADP and ATP. 2 sites bind either AMP or ATP, whereas a third site contains a tightly bound AMP that does not exchange. Under physiological conditions AMPK mainly exists in its inactive form in complex with ATP, which is much more abundant than AMP.

Post-translational modifications

Phosphorylated by ULK1 and ULK2; leading to negatively regulate AMPK activity and suggesting the existence of a regulatory feedback loop between ULK1, ULK2 and AMPK.

Images



Western blot - Anti-AMPK gamma 1 antibody [Y307] - BSA and Azide free (ab247251)

All lanes : Anti-AMPK gamma 1 antibody [Y307] (<u>ab32382</u>) at 1/1000 dilution

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: PRKAG1 (AMPK gamma 1) knockout HAP1 whole cell lysate

Lane 3: HeLa whole cell lysate

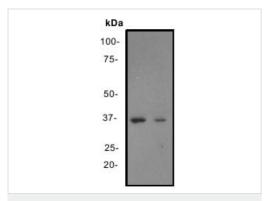
Lysates/proteins at 20 µg per lane.

Predicted band size: 38 kDa

This data was developed using <u>ab32382</u>, the same antibody clone in a different buffer formulation.

Lanes 1 - 3: Merged signal (red and green). Green - <u>ab32382</u> observed at 38 kDa. Red - loading control, Mouse anti-Vinculin, observed at 125 kDa.

ab32382 was shown to recognize AMPK gamma 1 in wild-type HAP1 cells as signal was lost at the expected MW in PRKAG1 (AMPK gamma 1) knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and PRKAG1 (AMPK gamma 1) knockout samples were subjected to SDS-PAGE. ab32382 and Mouse anti-Vinculin (ab9484 loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-AMPK gamma 1 antibody [Y307] - BSA and Azide free (ab247251)

All lanes : Anti-AMPK gamma 1 antibody [Y307] (<u>ab32382</u>) at 1/2000 dilution

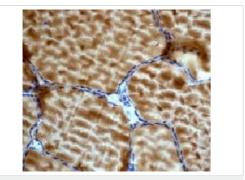
Lane 1 : Jurkat cell lysate

Lane 2 : HeLa cell lysate

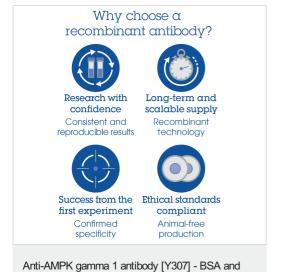
Predicted band size: 38 kDa **Observed band size:** 37 kDa

This data was developed using <u>ab32382</u>, the same antibody clone in a different buffer formulation.

This data was developed using <u>ab32382</u>, the same antibody clone in a different buffer formulation. This image shows paraffin embedded human skeletal muscle stained with 1/250 <u>ab23282</u>. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-AMPK gamma 1 antibody [Y307] - BSA and Azide free (ab247251)



Azide free (ab247251)

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