abcam

Product datasheet

Anti-AMPK gamma 1 antibody [Y308] ab32508

Recombinant RabMAb

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Overview

Product name	Anti-AMPK gamma 1 antibody [Y308]
Description	Rabbit monoclonal [Y308] to AMPK gamma 1
Host species	Rabbit
Specificity	ab32508 recognises 5'-AMP-activated protein kinase (AMPK).
Tested applications	**Suitable for:** WB, Flow Cyt, IP **Unsuitable for:** ICC or IHC
Species reactivity	**Reacts with:** Mouse, Rat, Human, African green monkey
Immunogen	Synthetic peptide within Human AMPK gamma 1 aa 300-400 (C terminal). The exact sequence is proprietary. (Peptide available as ab218345)
Positive control	Jurkat whole cell lysate (ab7899).
General notes	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents This product is a recombinant rabbit monoclonal antibody.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol, 0.05% BSA
Purity	IgG fraction
Clonality	Monoclonal
Clone number	Y308
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab32508** 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
WB	★★★★★	1/1000 - 1/10000. Detects a band of approximately 38 kDa (predicted molecular weight: 38 kDa).
Flow Cyt		1/20. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP		Use at an assay dependent concentration. PubMed: 23612997

Application notes Is unsuitable for ICC or IHC.

Target

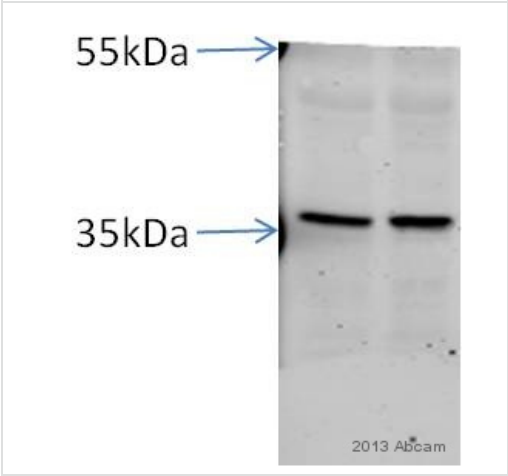
Function AMP/ATP-binding subunit of AMP-activated protein kinase (AMPK), an energy sensor protein kinase that plays a key role in regulating cellular energy metabolism. In response to reduction of intracellular ATP levels, AMPK activates energy-producing pathways and inhibits energy-consuming processes: 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 [Y308] (ab32508)

This image is courtesy of an anonymous Abreview

All lanes : Anti-AMPK gamma 1 antibody [Y308] (ab32508) at 1/1000 dilution

All lanes : HEK293 whole cell lysate

Lysates/proteins at 30 µg per lane.

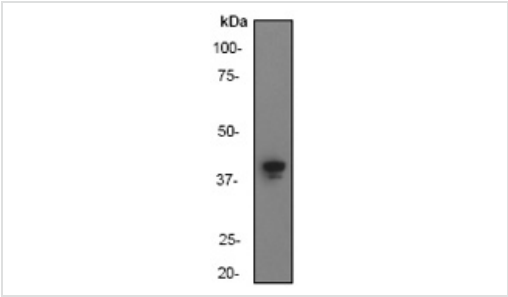
Secondary

All lanes : Alexa Fluor® 690-conjugated Goat anti-rabbit IgG polyclonal at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 38 kDa
Observed band size: 38 kDa

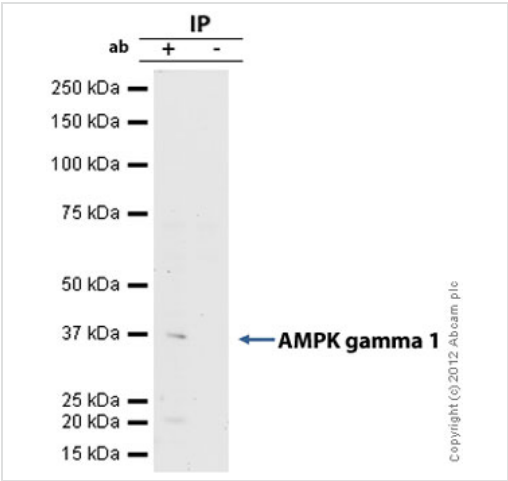
Exposure time: 5 minutes



Western blot - Anti-AMPK gamma 1 antibody [Y308] (ab32508)

Anti-AMPK gamma 1 antibody [Y308] (ab32508) at 1/1000 dilution + Jurkat cell lysate

Predicted band size: 38 kDa
Observed band size: 38 kDa



Immunoprecipitation - Anti-AMPK gamma 1 antibody [Y308] (ab32508)

AMPK gamma 1 was immunoprecipitated using 0.5mg Jurkat whole cell extract, 10ug of Rabbit monoclonal [Y308] to AMPK gamma 1and 50µl of protein G magnetic beads (lane 1). The antibody was incubated with the Protein G beads for 10min under agitation. No antibody was added to the control (lane 2). Jurkat whole cell extractdiluted in RIPA buffer was added to each sample and incubated for 10min under agitation. Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{o}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab32508. Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (ab99697). Bands: 37kDa: AMPK gamma 1.

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