# abcam

### Product datasheet

## Anti-AMPK gamma 1 antibody [Y308] ab32508



★★★★★ 7 Abreviews 24 References 5 Images

Overview

**Product name** Anti-AMPK gamma 1 antibody [Y308]

**Description** Rabbit monoclonal [Y308] to AMPK gamma 1

**Host species** Rabbit

Specificity This antibody recognises 5'-AMP-activated protein kinase (AMPK).

**Tested applications** Suitable for: Flow Cyt (Intra), IP, WB

Unsuitable for: ICC/IF or IHC

Reacts with: Human Species reactivity

Predicted to work with: Mouse, Rat, 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). Flow Cyt (intra): HeLa cells

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. 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 (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

**Clonality** Monoclonal

Clone number Y308
Isotype kgG

#### **Applications**

#### The Abpromise guarantee

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
Flow Cyt (Intra)		1/100. <b>ab172730</b> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
IP		Use at an assay dependent concentration.
WB	<b>★★★★★ (7)</b>	1/1000 - 1/10000. Detects a band of approximately 38 kDa (predicted molecular weight: 38 kDa).

**Application notes** 

Is unsuitable for ICC/IF 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

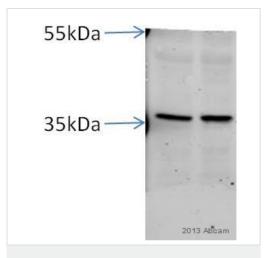
The AMPK pseudosubstrate motif resembles the sequence around sites phosphorylated on target proteins of AMPK, except the presence of a non-phosphorylatable residue in place of Ser. In the absence of AMP this pseudosubstrate sequence may bind to the active site groove on the alpha subunit (PRKAA1 or PRKAA2), 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

Phosphorylated by ULK1 and ULK2; leading to negatively regulate AMPK activity and suggesting

#### **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.

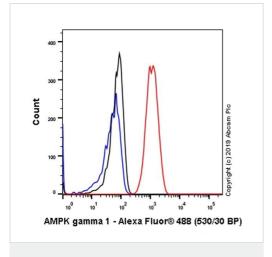
#### **Secondary**

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

Performed under reducing conditions.

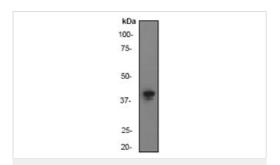
**Predicted band size:** 38 kDa **Observed band size:** 38 kDa

Exposure time: 5 minutes



Flow Cytometry (Intracellular) - Anti-AMPK gamma 1 antibody [Y308] (ab32508)

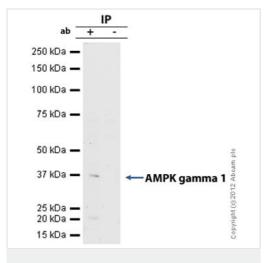
Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling AMPK gamma 1 with ab32508 at 1/100 dilution (1µg) (Red). Goat anti rabbit lgG (Alexa Fluor®488, **ab150077**) at 1/2000 dilution was used as the secondary antibody. Cells were fixed with4% paraformaldehyde. Rabbit monoclonal lgG (**ab172730**) was used as isotype control (Black). Unlabelled control: Cells without incubation with primary antibody and secondary antibody (Blue).



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

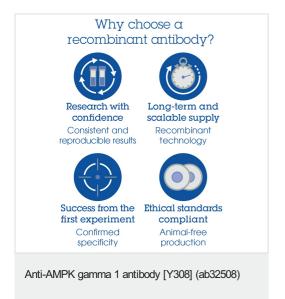
Anti-AMPK gamma 1 antibody [Y308] (ab32508) at 1/1000 dilution + Jurkat cell lysate

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



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

AMPK gamma 1 was immunoprecipitated using 1 mg Jurkat whole cell extract, 0.2 ug of Rabbit monoclonal [Y308] to AMPK gamma 1 and 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°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 lgG light chain (HRP) (ab99697). Bands: 37kDa: AMPK gamma 1.



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