abcam

Product datasheet

Anti-AMPK beta 1 antibody [Y367] ab32112

敲除验证 重组 RabMAb

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概述

产品名称	Anti-AMPK beta 1抗体[Y367]
描述	兔单克隆抗体[Y367] to AMPK beta 1
宿主	Rabbit
经测试应用	**适用于:** Flow Cyt (Intra), WB, IHC-P, IP, ICC/IF
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide within Human AMPK beta 1 aa 150-250. The exact sequence is proprietary. Database link: **Q9Y478**
阳性对照	HEK293 whole cell lysate (**ab7902**) can be used as a positive control in WB. NIH 3T3, HeLa, A431 and PC12, MCF-7 cell lysates. Human lung carcinoma tissue.
常规说明	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified
克隆	单克隆

克隆编号	Y367
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab32112于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/800. For unpurified use at 1/50. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		1/1000 - 1/5000. Predicted molecular weight: 30 kDa.
IHC-P	★★★★★ **(1)**	1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. The mouse and rat recommendation is based on the WB results. This antibody may not be suitable for IHC with mouse or rat samples.
IP		1/40. **For unpurified use at 1/80.**
ICC/IF		1/500.

靶标

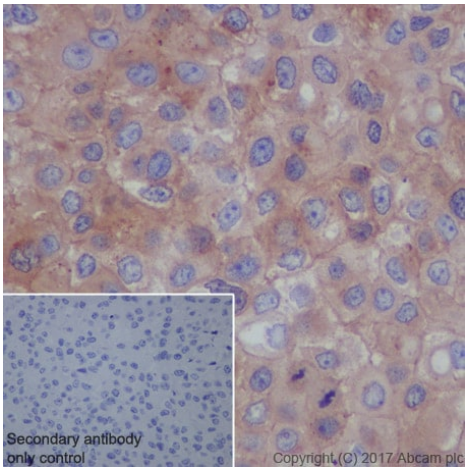
功能 Non-catalytic subunit of AMP-activated protein kinase (AMPK), an energy sensor protein kinase that plays a key role in regulating cellular energy metabolism. In response to reduction of intracellular ATP levels, AMPK activates energy-producing pathways and inhibits energy-consuming processes: inhibits 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. Beta non-catalytic subunit acts as a scaffold on which the AMPK complex assembles, via its C-terminus that bridges alpha (PRKAA1 or PRKAA2) and gamma subunits (PRKAG1, PRKAG2 or PRKAG3).

序列相似性 Belongs to the 5'-AMP-activated protein kinase beta subunit family.

结构域 The glycogen-binding domain may target AMPK to glycogen so that other factors like glycogen-bound debranching enzyme or protein phosphatases can directly affect AMPK activity.

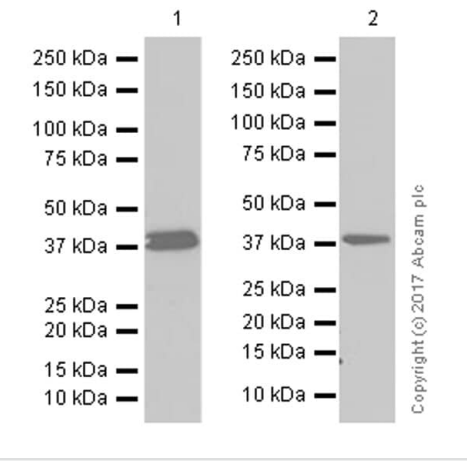
翻译后修饰 Phosphorylated when associated with the catalytic subunit (PRKAA1 or PRKAA2). Phosphorylated by ULK1; leading to negatively regulate AMPK activity and suggesting the existence of a regulatory feedback loop between ULK1 and AMPK.

图片



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AMPK beta 1 antibody [Y367] (ab32112)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human bladder carcinoma tissue sections labeling AMPK beta 1 with purified ab32112 at 1:1000 dilution (0.85 µg/ml). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using EDTA Buffer, pH9.0. Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



Western blot - Anti-AMPK beta 1 antibody [Y367] (ab32112)

All lanes : Anti-AMPK beta 1 antibody [Y367] (ab32112) at 1/5000 dilution (purified)

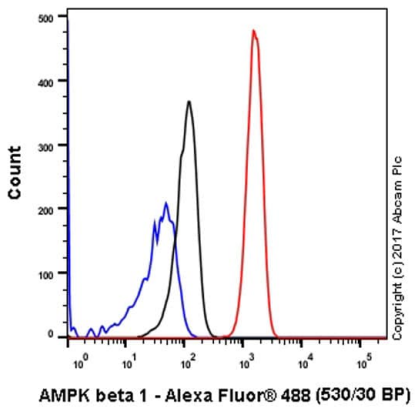
Lane 1 : Mouse brain lysates
Lane 2 : Rat brain lysates

Lysates/proteins at 15 µg per lane.

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

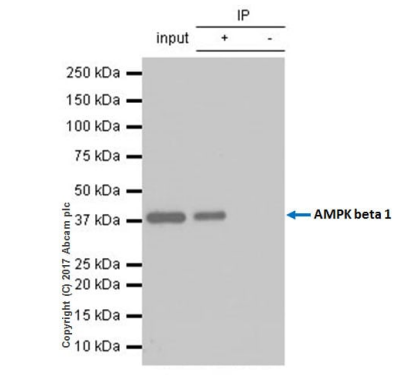
Predicted band size: 30 kDa
Observed band size: 38 kDa

Blocking and diluting buffer: 5% NFDM/TBST



Flow Cytometry (Intracellular) - Anti-AMPK beta 1 antibody [Y367] (ab32112)

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling AMPK beta 1 with purified ab32112 at 1/800 dilution (1 ug/ml) (red). Cells were fixed with 80% methanol. A Goat anti rabbit IgG (Alexa Fluorr® 488) secondary antibody was used at 1/2000 dilution. Isotype control - 0.1% Tween-20. Unlabeled control - Rabbit monoclonal IgG (Black).



Immunoprecipitation - Anti-AMPK beta 1 antibody [Y367] (ab32112)

ab32112 (purified) at 1:40 dilution (2ug) immunoprecipitating AMPK beta 1 in NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates.

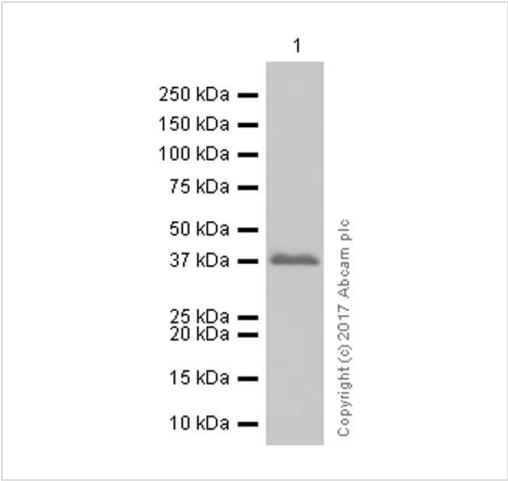
Lane 1 (input): NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates 10ug

Lane 2 (+): ab32112 & NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32112 in NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.



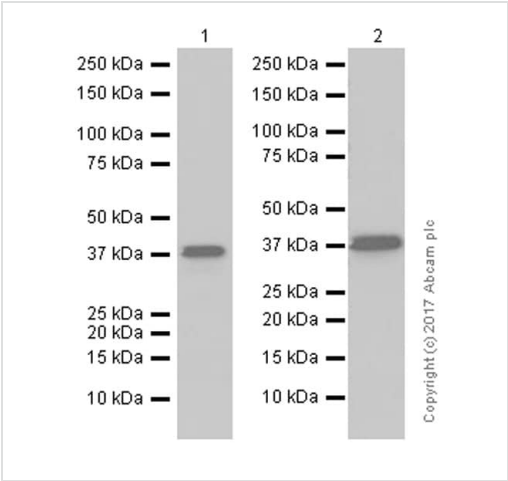
Western blot - Anti-AMPK beta 1 antibody [Y367] (ab32112)

Anti-AMPK beta 1 antibody [Y367] (ab32112) at 1/20000 dilution (purified) + HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates at 20 µg

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

Predicted band size: 30 kDa
Observed band size: 38 kDa

Blocking and diluting buffer: 5% NFDM/TBST



Western blot - Anti-AMPK beta 1 antibody [Y367] (ab32112)

All lanes : Anti-AMPK beta 1 antibody [Y367] (ab32112) at 1/5000 dilution (purified)

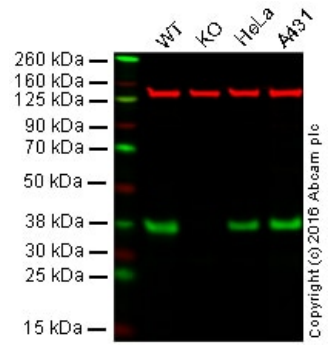
Lane 1 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates
Lane 2 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysates

Lysates/proteins at 15 µg per lane.

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

Predicted band size: 30 kDa
Observed band size: 38 kDa

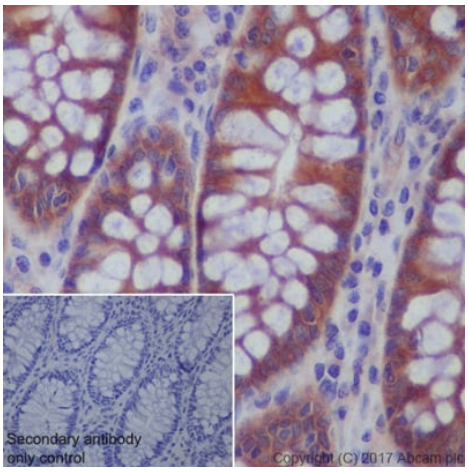
Blocking and diluting buffer: 5% NFDM/TBST



Western blot - Anti-AMPK beta 1 antibody [Y367] (ab32112)

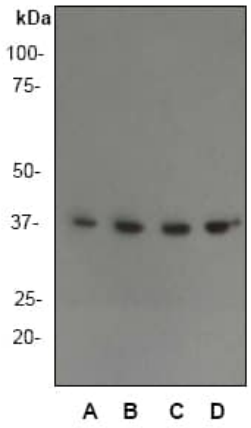
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: AMPK beta 1 knockout HAP1 cell lysate
Lane 3: HeLa cell lysate
Lane 4: A431 cell lysate
Lanes 1 - 4: Merged signal (red and green). Green - Unpurified ab32112 observed at 38 kDa. Red - loading control, **ab18058**, observed at 124 kDa.

Unpurified ab32112 was shown to specifically react with AMPK beta 1 when AMPK beta 1 knockout samples were used. Wild-type and AMPK beta 1 knockout samples were subjected to SDS-PAGE. ab32112 and **ab18058** (loading control to Vinculin) were both diluted 1/10000 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 1/10000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AMPK beta 1 antibody [Y367] (ab32112)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human colon tissue sections labeling AMPK beta 1 with purified ab32112 at 1:1000 dilution (0.85 µg/ml). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using EDTA Buffer, pH9.0. Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.

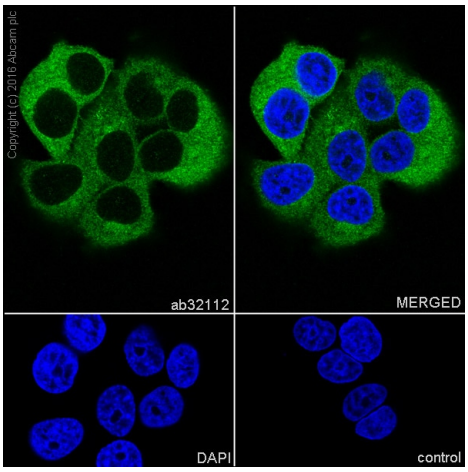


Western blot - Anti-AMPK beta 1 antibody [Y367] (ab32112)

All lanes : Anti-AMPK beta 1 antibody [Y367] (ab32112) at 1/5000 dilution (unpurified)

Lane 1 : (A) : NIH 3T3.
Lane 2 : (B) : Hela.
Lane 3 : (C) : A431.
Lane 4 : (D) : PC-12.

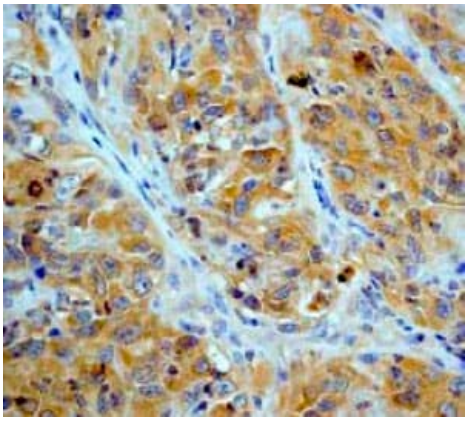
Predicted band size: 30 kDa
Observed band size: 38 kDa



Immunocytochemistry/ Immunofluorescence - Anti-AMPK beta 1 antibody [Y367] (ab32112)

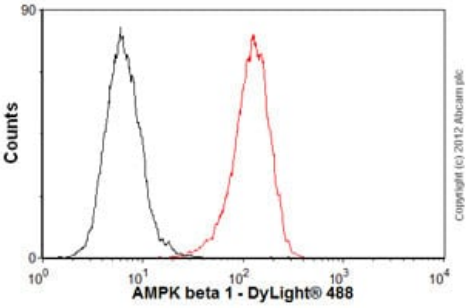
Immunocytochemistry/Immunofluorescence analysis of MCF-7 cells labelling AMPK beta 1 with purified ab32112 at 1/500. Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% tritonX-100. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (**ab150077**) at 1/1000 dilution was used as the secondary antibody. Nuclei counterstained with DAPI (blue).

Secondary Only Control: PBS was used instead of the primary antibody as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AMPK beta 1 antibody [Y367] (ab32112)

Unpurified ab32112 at a 1:100 dilution staining AMPK beta 1 in human lung carcinoma, using Immunohistochemistry, Paraffin Embedded Tissue.



Flow Cytometry (Intracellular) - Anti-AMPK beta 1 antibody [Y367] (ab32112)

Overlay histogram showing HeLa cells stained with unpurifiedab32112 (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 (ab32112, 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 (monclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

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.cn/abpromise** or contact our technical team.

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