abcam

Product datasheet

Anti-AMPK alpha 1 antibody [EPR24413-70] ab271188

敲除验证 重组 RabMAb

1 References 11 图像

概述

产品名称	Anti-AMPK alpha 1抗体[EPR24413-70]
描述	兔单克隆抗体[EPR24413-70] to AMPK alpha 1
宿主	Rabbit
特异性	IHC application does not react with Human species.
经测试应用	**适用于:** WB, Flow Cyt (Intra), IHC-P, IP
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: Wild-type HAP1, MCF7, HeLa , Wild-type RAW264.7, NIH/3T3, Neuro-2a, C6, Mouse liver, Mouse brain, Mouse heart, Rat brain, Rat heart, His-tagged mouse AMPK alpha 1 recombinant protein and His-tagged mouse AMPK alpha 2 recombinant protein lysates. IHC-P: Mouse cerebrum and Rat cerebrum tissues. Flow Cyt: Wild-type HAP1, Neuro-2a and C6 cells. IP: Neuro-2a and C6 cells.
常规说明	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**.

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: 7.2 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	EPR24413-70
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab271188于以下的经测试应用**

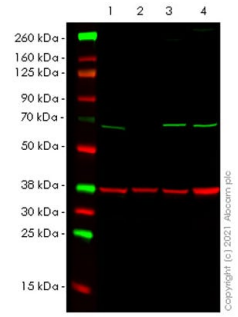
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		1/1000. Predicted molecular weight: 64 kDa.
Flow Cyt (Intra)		1/50.
IHC-P		1/100. IHC application does not react with Human species.
IP		1/30.

靶标

功能	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.
序列相似性	Belongs to the protein kinase superfamily. CAMK Ser/Thr protein kinase family. SNF1 subfamily. Contains 1 protein kinase domain.

图片



Western blot - Anti-AMPK alpha 1 antibody [EPR24413-70] (ab271188)

Lanes 2-4 : Anti-AMPK alpha 1 antibody [EPR24413-70] (ab271188) at 1/1000 dilution

Lane 1 : Wild-type HAP1 (human chronic myelogenous leukemia near-haploid cell) whole cell lysate
Lane 2 : AMPK alpha 1 knockout HAP1 whole cell lysate
Lane 3 : MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate
Lane 4 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (**ab216773**) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (**ab216776**) at 1/10000 dilution

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

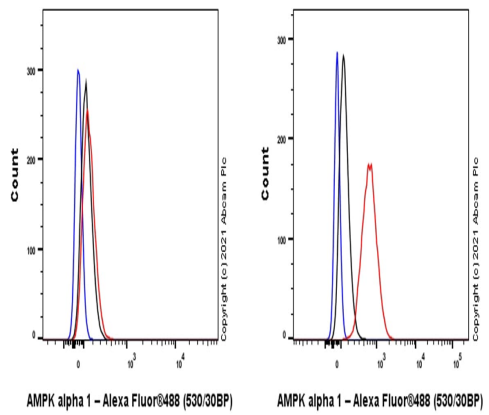
Blocking and diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS.
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

False colour image of Western blot: Anti-AMPK alpha 1 antibody [EPR24413-70] (ab271188) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red.

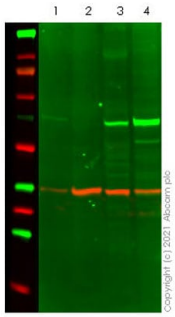
In Western blot, ab271188 was shown to bind specifically to AMPK alpha 1. A band was observed at 63 kDa in wild-type HAP1 cell lysates with no signal observed at this size in AMPK alpha 1 knockout cell lysates. To generate this image, wild-type and AMPK alpha 1 knockout HAP1 cell lysates were analyzed. First, samples were run on an SDS-PAGE gel then transferred onto an immobilon-FL PVDF membrane. Membranes were blocked in Intercept®(TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS 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 1/10000 dilution.



Flow Cytometry (Intracellular) - Anti-AMPK alpha 1 antibody (ab271188)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Wild-type HAP1 (human chronic myelogenous leukemia near-haploid cell, Right) / PRAKK1 knockout HAP1(Left) cells labelling AMPK alpha 1 with ab271188 at 1/50 dilution (1µg) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody.



Western blot - Anti-AMPK alpha 1 antibody [EPR24413-70] (ab271188)

All lanes : Anti-AMPK alpha 1 antibody [EPR24413-70] (ab271188) at 1/1000 dilution

Lane 1 : Wild-type RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
Lane 2 : AMPK alpha 1 knockout RAW 264.7 whole cell lysate
Lane 3 : Mouse liver tissue lysate
Lane 4 : Neuro-2a (mouse neuroblastoma neuroblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (**ab216773**) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (**ab216776**) at 1/10000 dilution

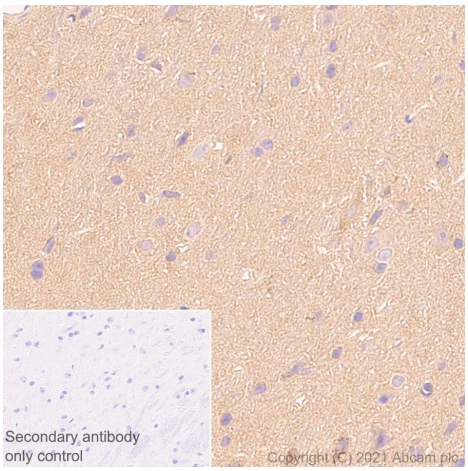
Predicted band size: 64 kDa
Observed band size: 63 kDa

Blocking and diluting buffer and concentration: Intercept®(TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS.
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

False colour image of Western blot: Anti-AMPK alpha 1 antibody [EPR24413-70] (ab271188) 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, ab271188 was shown to bind specifically to AMPK alpha 1. A band was observed at 63 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 AMPK alpha 1 knockout RAW 264.7 cell lysates were analyzed. First, samples were run on an SDS-PAGE gel then transferred onto an immobilon-FL PVDF membrane. Membranes were blocked in Intercept®(TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS 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 1/10000 dilution.

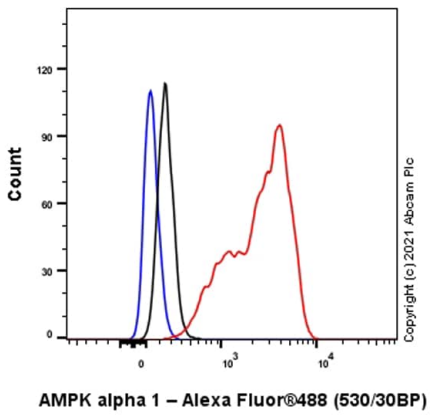


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AMPK alpha 1 antibody (ab271188)

Immunohistochemical analysis of paraffin-embedded Mouse cerebrum tissue labelling AMPK alpha 1 with ab271188 at 1/100 (4.91 ug/ml) followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection). Positive staining on mouse cerebrum (PMID: 25538235) (PMID: 10098881). The section was incubated with ab271188 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

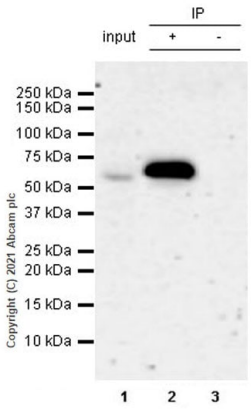
Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection) was used.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Flow Cytometry (Intracellular) - Anti-AMPK alpha 1 antibody (ab271188)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Neuro-2a (Mouse neuroblastoma neuroblast) cells labelling AMPK alpha 1 with ab271188 at 1/50 dilution (1µg) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-AMPK alpha 1 antibody (ab271188)

AMPK alpha 1 was immunoprecipitated from 0.35 mg Neuro-2a (mouse neuroblastoma neuroblast) whole cell lysate with ab271188 at 1/30 dilution (2 µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab271188 at 1/2000 dilution. VeriBlot for IP secondary antibody(HRP)(**ab131366**) was used at 1/5000 dilution.

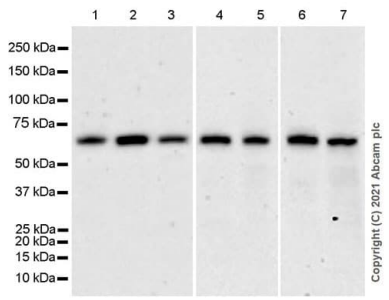
Lane 1: Neuro-2a (mouse neuroblastoma neuroblast) whole cell lysate 10 µg

Lane 2: ab271188 IP in Neuro-2a whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab271188 in Neuro-2a whole cell lysate

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

Exposure time: 3 minutes



Western blot - Anti-AMPK alpha 1 antibody [EPR24413-70] (ab271188)

All lanes : Anti-AMPK alpha 1 antibody [EPR24413-70] (ab271188) at 1/1000 dilution

Lane 1 : NIH/3T3 (mouse embryonic fibroblast) whole cell lysate
Lane 2 : Neuro-2a (mouse neuroblastoma neuroblast) whole cell lysate
Lane 3 : C6 (rat glial tumor glial cell) whole cell lysate
Lane 4 : Mouse brain tissue lysate
Lane 5 : Mouse heart tissue lysate
Lane 6 : Rat brain tissue lysate
Lane 7 : Rat heart tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

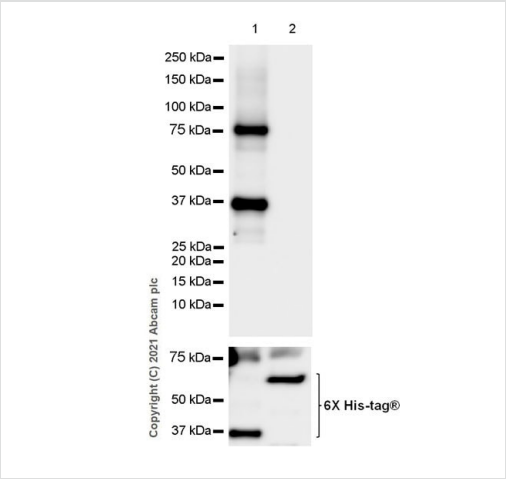
Predicted band size: 64 kDa
Observed band size: 63 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Lysates/proteins at 20 µg per lane.

Lane 4-7: This blot was developed using a higher sensitivity ECL substrate.

Exposure time: 3 minutes



Western blot - Anti-AMPK alpha 1 antibody [EPR24413-70] (ab271188)

All lanes : Anti-AMPK alpha 1 antibody [EPR24413-70] (ab271188) at 1/1000 dilution

Lane 1 : His-tagged mouse AMPK alpha 1 recombinant protein
Lane 2 : His-tagged mouse AMPK alpha 2 recombinant protein

Lysates/proteins at 10 µg per lane.

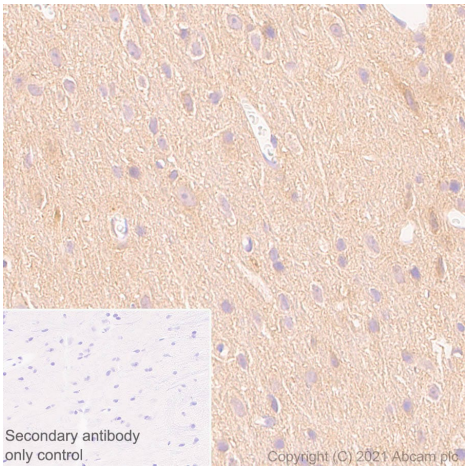
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

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

Blocking and diluting buffer and concentration: 5% NFDM/TBST

This antibody has no cross-reaction with mouse AMPK alpha 2. Both recombinant proteins were made in-house and expressed from E.coli expression systems.

Exposure time: 15 seconds



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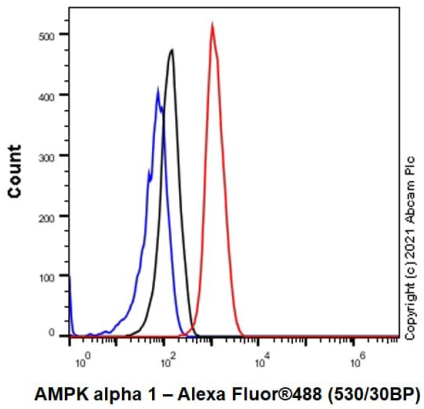

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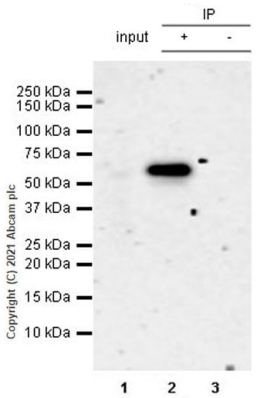

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Lane 2: ab271188 IP in C6 whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab271188 in C6 whole cell lysate

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

Exposure time: 3 minutes

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