abcam

ab288572

Anti-AMPK alpha 1 antibody [EPR24413-70] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR24413-70
Purification technique	Affinity purification Protein A
Specificity	IHC application does not react with Human species.
Concentration	1.025 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Mouse, Rat

Dilution info	-
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Recombinant full length protein
Dilution info	-
Notes	-

WB

Tested

Species	Recombinant full length protein, Mouse, Rat, Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Mouse, Rat
Dilution info	-
Notes	-

Not recommended

Species	Human, Recombinant full length protein
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

Not recommended

Species	Recombinant full length protein
Dilution info	-
Notes	-

Target data

See full target information PRKAA1

Function

Catalytic subunit of AMP-activated protein kinase (AMPK), an energy sensor protein kinase that plays a key role in regulating cellular energy metabolism (PubMed:17307971, PubMed:17712357, PubMed:24563466, PubMed:31492851, PubMed:37821951, PubMed:40233740). 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 (PubMed:17307971, PubMed:17712357). AMPK acts via direct phosphorylation of metabolic enzymes, and by longer-term effects via phosphorylation of transcription regulators (PubMed:17307971, PubMed:17712357). Regulates lipid synthesis by phosphorylating and inactivating lipid metabolic enzymes such as ACACA, ACACB, GYS1, HMGCR and LIPE; regulates fatty acid and cholesterol synthesis by phosphorylating acetyl-CoA carboxylase (ACACA and ACACB) and hormone-sensitive lipase (LIPE) enzymes, respectively (By similarity). Promotes lipolysis of lipid droplets by mediating phosphorylation of isoform 1 of CHKA (CHKalpha2) (PubMed:34077757). Regulates insulin-signaling and glycolysis by phosphorylating IRS1, PFKFB2 and PFKFB3 (By similarity). AMPK stimulates glucose uptake in muscle by increasing the translocation of the glucose transporter SLC2A4/GLUT4 to the plasma membrane, possibly by mediating phosphorylation of TBC1D4/AS160 (By similarity). Regulates transcription and chromatin structure by phosphorylating transcription regulators involved in energy metabolism such as CRTC2/TORC2, FOXO3, histone H2B, HDAC5, MEF2C, MLXIPL/ChREBP, EP300, HNF4A, p53/TP53, SREBF1, SREBF2 and PPARGC1A (PubMed:11518699, PubMed:11554766, PubMed:15866171, PubMed:17711846, PubMed:18184930). Acts as a key regulator of glucose homeostasis in liver by phosphorylating CRTC2/TORC2, leading to CRTC2/TORC2 sequestration in the cytoplasm (By similarity). In response to stress, phosphorylates 'Ser-36' of histone H2B (H2BS36ph), leading to promote transcription (By similarity). Acts as a key regulator of cell growth and proliferation by phosphorylating FNIP1, TSC2, RPTOR,

WDR24 and ATG1/ULK1: in response to nutrient limitation, negatively regulates the mTORC1 complex by phosphorylating RPTOR component of the mTORC1 complex and by phosphorylating and activating TSC2 (PubMed:14651849, PubMed:18439900, PubMed:20160076, PubMed:21205641). Also phosphorylates and inhibits GATOR2 subunit WDR24 in response to nutrient limitation, leading to suppress glucose-mediated mTORC1 activation (PubMed:36732624). In response to energetic stress, phosphorylates FNIP1, inactivating the non-canonical mTORC1 signaling, thereby promoting nuclear translocation of TFEB and TFE3, and inducing transcription of lysosomal or autophagy genes (PubMed:37079666). In response to nutrient limitation, promotes autophagy by phosphorylating and activating ATG1/ULK1 (PubMed:21205641). In that process, it also activates WDR45/WIPI4 (PubMed:28561066). Phosphorylates CASP6, thereby preventing its autoprocessing and subsequent activation (PubMed:32029622). In response to nutrient limitation, phosphorylates transcription factor FOXO3 promoting FOXO3 mitochondrial import (By similarity). Also acts as a regulator of cellular polarity by remodeling the actin cytoskeleton; probably by indirectly activating myosin (PubMed:17486097). AMPK also acts as a regulator of circadian rhythm by mediating phosphorylation of CRY1, leading to destabilize it (By similarity). May regulate the Wnt signaling pathway by phosphorylating CTNNB1, leading to stabilize it (By similarity). Also has tau-protein kinase activity: in response to amyloid beta A4 protein (APP) exposure, activated by CAMKK2, leading to phosphorylation of MAPT/TAU; however the relevance of such data remains unclear in vivo (By similarity). Also phosphorylates CFTR, EEF2K, KLC1, NOS3 and SLC12A1 (PubMed:12519745, PubMed:20074060). Regulates hepatic lipogenesis. Activated via SIRT3, represses sterol regulatory element-binding protein (SREBP) transcriptional activities and ATP-consuming lipogenesis to restore cellular energy balance. Upon stress, regulates mitochondrial fragmentation through phosphorylation of MTFR1L (PubMed:36367943). Phosphorylates ALDH7A1 in response to cellular stress, such as hypoxia or ferroptotic stress, promoting ALDH7A1 recruitment to membranes (PubMed:31492851, PubMed:40233740).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C

Notes

ab288572 is the carrier-free version of ab271188

Patented technology

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?

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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

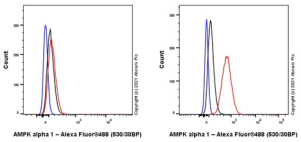
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

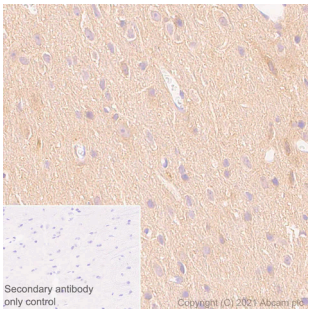
12 product images



Flow Cytometry (Intracellular) - Anti-AMPK alpha 1 antibody [EPR24413-70] - BSA and Azide free (ab288572)

AMPK alpha 1 Flow Cytometry (Intracellular) staining using rabbit Anti-AMPK alpha 1 antibody

This data was developed using ab271188, the same antibody clone in a different buffer formulation.
Flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized Wild-type HAP1 (human chronic myelogenous leukemia near-haploid cell, Right) / AMPK alpha 1 knockout HAP1(Left) cells labelling AMPK alpha 1 with ab271188 at 1/50 dilution (1ug) (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.



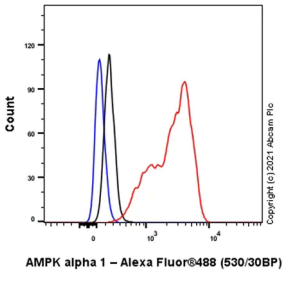
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AMPK alpha 1 antibody [EPR24413-70] - BSA and Azide free (ab288572)

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

This data was developed using ab271188, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded Rat 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 rat 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.

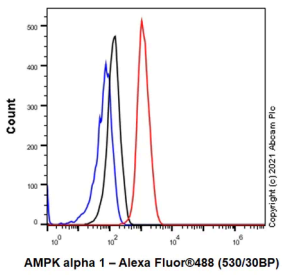
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Flow Cytometry (Intracellular) - Anti-AMPK alpha 1 antibody [EPR24413-70] - BSA and Azide free (ab288572)

AMPK alpha 1 Flow Cytometry (Intracellular) staining using rabbit Anti-AMPK alpha 1 antibody

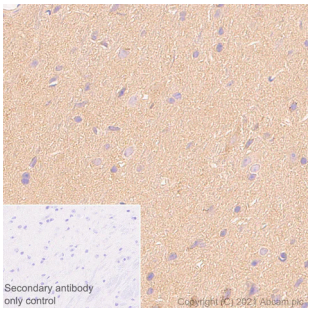
This data was developed using ab271188, the same antibody clone in a different buffer formulation.
Flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized Neuro-2a (Mouse neuroblastoma neuroblast) cells labelling AMPK alpha 1 with ab271188 at 1/50 dilution (1ug) (Red) compared 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.



Flow Cytometry (Intracellular) - Anti-AMPK alpha 1 antibody [EPR24413-70] - BSA and Azide free (ab288572)

AMPK alpha 1 Flow Cytometry (Intracellular) staining using rabbit Anti-AMPK alpha 1 antibody

This data was developed using ab271188, the same antibody clone in a different buffer formulation.
Flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized C6 (rat glial tumor glial cell) cells labelling AMPK alpha 1 with ab271188 at 1/50 dilution (1ug) (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.



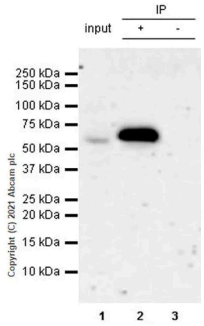
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AMPK alpha 1 antibody [EPR24413-70] - BSA and Azide free (ab288572)

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

This data was developed using ab271188, the same antibody clone in a different buffer formulation.
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



Immunoprecipitation - Anti-AMPK alpha 1 antibody [EPR24413-70] - BSA and Azide free (ab288572)

This data was developed using ab271188, the same antibody clone in a different buffer formulation.
AMPK alpha 1 was immunoprecipitated from 0.35 mg Neuro-2a (mouse neuroblastoma neuroblast) whole cell lysate with ab271188 at 1/30 dilution (2ug 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 ug

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

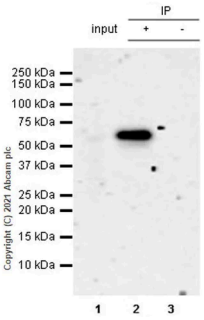
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes

All lanes:
Immunoprecipitation - Anti-AMPK alpha 1 antibody [EPR24413-70] (ab271188)

Predicted band size: 64 kDa

Observed band size: 63 kDa



Immunoprecipitation - Anti-AMPK alpha 1 antibody [EPR24413-70] - BSA and Azide free (ab288572)

This data was developed using ab271188, the same antibody clone in a different buffer formulation.
AMPK alpha 1 was immunoprecipitated from 0.35 mg C6 (rat glial tumor glial cell) whole cell lysate with ab271188 at 1/30 dilution (2ug 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: C6 (rat glial tumor glial cell) whole cell lysate 10 ug

Lane 2: ab271188 IP in C6 whole cell lysate

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

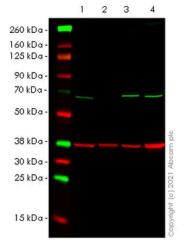
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes

All lanes:
Immunoprecipitation - Anti-AMPK alpha 1 antibody [EPR24413-70] (ab271188)

Predicted band size: 64 kDa

Observed band size: 63 kDa



Western blot - Anti-AMPK alpha 1 antibody [EPR24413-70] - BSA and Azide free (ab288572)

AMPK alpha 1 Western blot staining using rabbit Anti-AMPK alpha 1 antibody

This data was developed using ab271188, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS 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.
Lane 1:
Western blot - Anti-AMPK alpha 1 antibody [EPR24413-70] (ab271188)

Lanes 2 - 4:
Western blot - 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 at 20 µg

Lane 2:
AMPK alpha 1 knockout HAP1 whole cell lysate at 20 µg

Lane 3:
MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

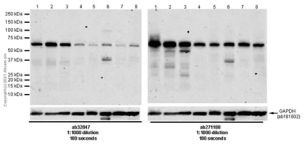
Lane 4:
HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

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



Western blot - Anti-AMPK alpha 1 antibody [EPR24413-70] - BSA and Azide free (ab288572)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab271188).
Blocking and diluting buffer and concentration: 5% NFDM /TBST.

ab181602 was used as a GAPDH loading control at 1/1000000 dilution.

This blot was developed using a higher sensitivity ECL substrate.

Compared with ab32047, ab271188 has higher sensitivity, we recommend ab271188 as an alternative for testing AMPK alpha 1 in mouse and rat samples in western blot.

Lanes 1 - 8:
Western blot - Anti-AMPK alpha 1 antibody [Y365] (ab32047) at 1/1000 dilution

Lanes 1 - 8:
Western blot - Anti-AMPK alpha 1 antibody [EPR24413-70] (ab271188) at 1/1000 dilution

Lane 1:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

Lane 2:
Neuro-2a (mouse neuroblastoma neuroblast) whole cell lysate at 20 µg

Lane 3:

C6 (rat glial tumor glial cell) whole cell lysate at 20 µg

Lane 4:
Mouse brain tissue lysate at 20 µg

Lane 5:
Mouse heart tissue lysate at 20 µg

Lane 6:
Mouse skeletal muscle tissue lysate at 20 µg

Lane 7:
Rat brain tissue lysate at 20 µg

Lane 8:
Rat heart tissue lysate at 20 µg

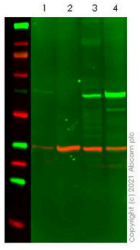
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Observed band size: 64 kDa

Exposure time: 180s



Western blot - Anti-AMPK alpha 1 antibody [EPR24413-70] - BSA and Azide free (ab288572)

AMPK alpha 1 Western blot staining using rabbit Anti-AMPK alpha 1 antibody

This data was developed using ab271188, the same antibody clone in a different buffer formulation.
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.

All lanes:
Western blot - 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 at 20 µg

Lane 2:
AMPK alpha 1 knockout RAW 264.7 whole cell lysate at 20 µg

Lane 3:
Mouse liver tissue lysate at 20 µg

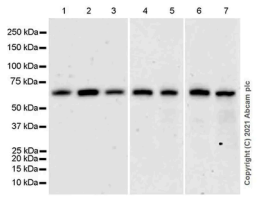
Lane 4:
Neuro-2a (mouse neuroblastoma neuroblast) whole cell lysate at 20 µg

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



Western blot - Anti-AMPK alpha 1 antibody [EPR24413-70] - BSA and Azide free (ab288572)

AMPK alpha 1 Western blot staining using rabbit Anti-AMPK alpha 1 antibody

This data was developed using ab271188, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Lysates/proteins at 20 µg per lane.

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

Exposure time: 3 minutes

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

Lane 1:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

Lane 2:
Neuro-2a (mouse neuroblastoma neuroblast) whole cell lysate at 20 µg

Lane 3:
C6 (rat glial tumor glial cell) whole cell lysate at 20 µg

Lane 4:
Mouse brain tissue lysate at 20 µg

Lane 5:
Mouse heart tissue lysate at 20 µg

Lane 6:
Rat brain tissue lysate at 20 µg

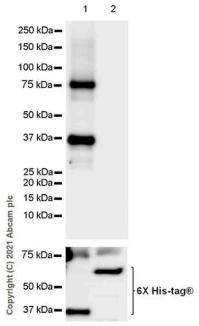
Lane 7:
Rat heart tissue lysate at 20 µg

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



Western blot - Anti-AMPK alpha 1 antibody [EPR24413-70] - BSA and Azide free (ab288572)

This data was developed using ab271188, the same antibody clone in a different buffer formulation.
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

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

Lane 1:
His-tagged mouse AMPK alpha 1 recombinant protein at 10 µg

Lane 2:
His-tagged mouse AMPK alpha 2 recombinant protein at 10 µg

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

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.