

Product datasheet

Anti-AMPK alpha 1 (phospho T183) + AMPK alpha 2 (phospho T172) antibody ab23875

★★★★★ [3 Abreviews](#) [72 References](#) [4 Images](#)

Overview

Product name	Anti-AMPK alpha 1 (phospho T183) + AMPK alpha 2 (phospho T172) antibody
Description	Rabbit polyclonal to AMPK alpha 1 (phospho T183) + AMPK alpha 2 (phospho T172)
Host species	Rabbit
Specificity	ab23875 recognises the phosphorylated forms of AMPK alpha 1 (T183) and AMPK alpha 2 (T172).
Tested applications	Suitable for: WB, IHC-P, Flow Cyt, ICC/IF
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide corresponding to Human AMPK alpha 1 (phospho T183). Also within AMPK alpha 2 (phospho T172).
Positive control	Insulin treated CHO T cells, Insulin treated 3T3L1, Metformin treated L6 myoblast cells. Metformin treated HepG2 cells (10 mM for 24 hr).
General notes	<p>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.</p> <p>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</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term.
Storage buffer	pH: 7.30 Preservative: 0.05% Sodium azide Constituents: PBS, 50% Glycerol, 0.1% BSA
Purity	Immunogen affinity purified
Purification notes	ab23875 was purified from rabbit serum by sequential epitope specific chromatography. The

antibody has been negatively preadsorbed using a non phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non phosphorylated AMPK. The final product is generated by affinity chromatography using a AMPK derived peptide that is phosphorylated at threonine 172.

Clonality Polyclonal
Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab23875 in the following tested applications.

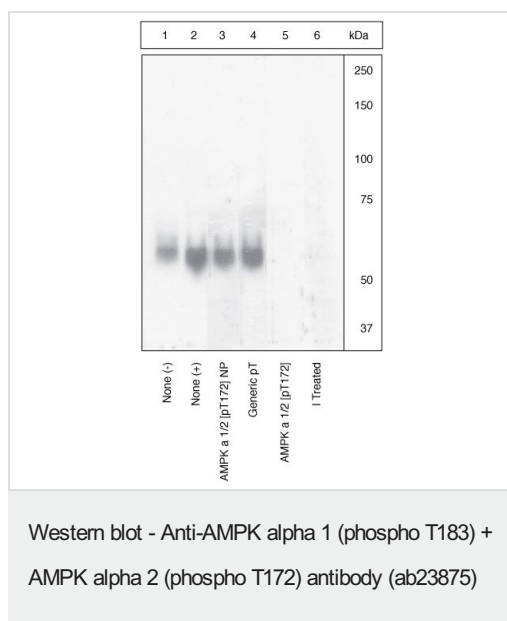
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000. Predicted molecular weight: 62 kDa.
IHC-P		1/10 - 1/50.
Flow Cyt		1/20.
ICC/IF		1/250.

Target

Cellular localization AMPK alpha 2: Cytoplasm. Nucleus. In response to stress, recruited by p53/TP53 to specific promoters.

Images



All lanes : Anti-AMPK alpha 1 (phospho T183) + AMPK alpha 2 (phospho T172) antibody (ab23875)

Lane 1 : Lysates prepared from HepG2 cells left unstimulated with 3% BSA-TBST buffer and no peptide

Lane 2 : Lysates prepared from HepG2 cells stimulated with Metformin with 3% BSA-TBST buffer and no peptide

Lane 3 : Lysates prepared from HepG2 cells stimulated with Metformin with 3% BSA-TBST buffer and the non-phosphopeptide corresponding to the immunogen

Lane 4 : Lysates prepared from HepG2 cells stimulated with Metformin with 3% BSA-TBST buffer and a generic phospho-threonine-containing peptide

Lane 5 : Lysates prepared from HepG2 cells stimulated with Metformin with 3% BSA-TBST buffer and the phosphopeptide immunogen

Lane 6 : Lysates prepared from HepG2 cells stimulated with Metformin and treated with lambda phosphatase with 3% BSA-TBST buffer

Secondary

All lanes : goat F(ab')₂ anti rabbit IgG HRP conjugate

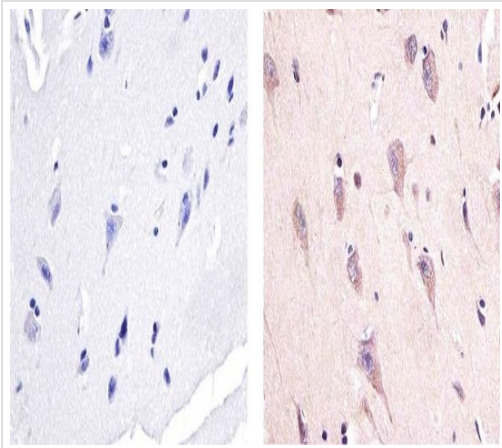
Predicted band size: 62 kDa

Observed band size: 60 kDa

Exposure time: 2 hours

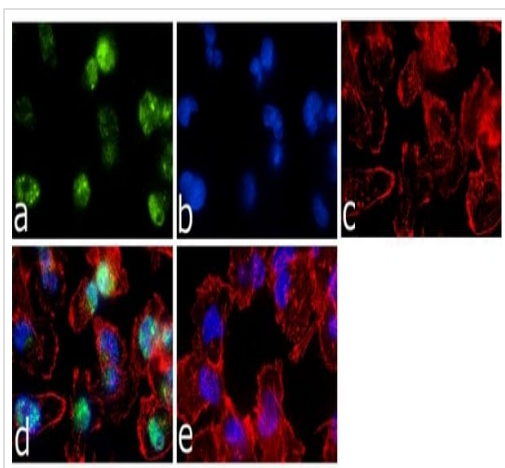
Lysates were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF, treated or not with lambda phosphatase, blocked with a 3% BSA-TBST buffer for one hour at room temperature, incubated with relevant peptides (see below) and incubated with the AMPK alpha 1/2 [pT 172] antibody for two hours at room temperature in 3% BSA-TBST buffer.

After washing, membranes were incubated with goat F(ab')₂ antirabbit IgG HRP conjugate and bands were detected using the Pierce SuperSignal™ method.



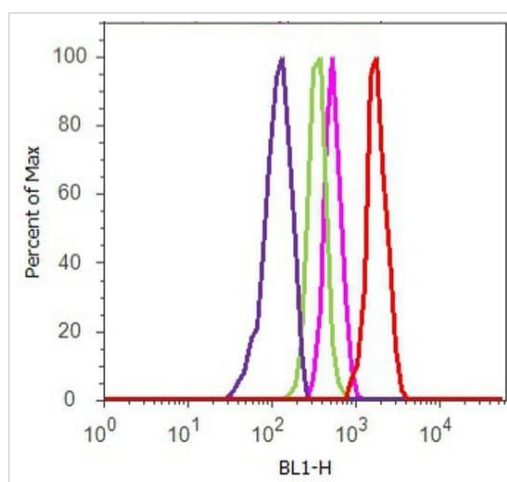
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AMPK alpha 1 (phospho T183) + AMPK alpha 2 (phospho T172) antibody (ab23875)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human brain tissue sections labeling AMPK alpha 1 (phospho T183) + AMPK alpha 2 (phospho T172) with ab23875 (right) compared to a negative control without primary antibody (left). To expose target proteins, antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H₂O₂-methanol for 15 min at room temperature, washed with ddH₂O and PBS, and then probed with AMPK alpha 1 (phospho T183) + AMPK alpha 2 (phospho T172) antibody (ab23875) diluted in 3% BSA-PBS at a dilution of 1/20 overnight at 4°C in a humidified chamber. Tissues were washed extensively in PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.



Immunocytochemistry/ Immunofluorescence - Anti-AMPK alpha 1 (phospho T183) + AMPK alpha 2 (phospho T172) antibody (ab23875)

Immunocytochemistry/ Immunofluorescence analysis of 70% confluent log phase MDA-MB-231 cells labeling AMPK alpha 1 (phospho T183) + AMPK alpha 2 (phospho T172) with ab23875. The cells were fixed with 4% paraformaldehyde for 15 minutes, permeabilized with 0.25% Triton™ X-100 for 10 minutes, and blocked with 5% BSA for 1 hour at room temperature. The cells were labeled with Anti-AMPK alpha 1 (phospho T183) + AMPK alpha 2 (phospho T172) antibody (ab23875) at 1ug/mL in 1% BSA and incubated for 3 hours at room temperature and then labeled with Goat anti-Rabbit IgG (H+L) secondary antibody, Alexa Fluor® 488 conjugate at a dilution of 1/2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with mountant with DAPI. F-actin (Panel c: red) was stained with Alexa Fluor® 555 Rhodamine Phalloidin, 1/300. Panel d is a merged image showing Nuclear localization. Panel e is a no primary antibody control. The images were captured at 60X magnification.



Flow Cytometry - Anti-AMPK alpha 1 (phospho T183) + AMPK alpha 2 (phospho T172) antibody (ab23875)

Flow Cytometry analysis of MDA-MB-231 cells labeling AMPK alpha 1 (phospho T183) + AMPK alpha 2 (phospho T172) with ab23875. Cells were fixed with 70% ethanol for 10 minutes, permeabilized with 0.25% Triton™ X-100 for 20 minutes, and blocked with 5% BSA for 30 minutes at room temperature. Cells were labeled with Anti-AMPK alpha 1 (phospho T183) + AMPK alpha 2 (phospho T172) antibody (ab23875, red) or with rabbit isotype control (pink) at 3-5 ug/million cells in 2.5% BSA. After incubation at room temperature for 2 hours, the cells were labeled with Alexa Fluor® 488 Goat Anti-Rabbit Secondary Antibody at a dilution of 1/400 for 30 minutes at room temperature. The representative 10,000 cells were acquired and analyzed for each sample using an Attune® Acoustic Focusing Cytometer. The purple histogram represents unstained control cells and the green histogram represents no-primary-antibody control.

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