**Product datasheet**

**Anti-AMPK alpha 1 antibody [Y365] ab32047**

**Overview**

**Product name**  
Anti-AMPK alpha 1 antibody [Y365]

**Description**  
Rabbit monoclonal [Y365] to AMPK alpha 1

**Host species**  
Rabbit

**Specificity**  
This antibody is specific for human AMPK alpha 1. This antibody shows low affinity on mouse and rat samples.

**Tested applications**  
Suitable for: ICC/IF, Flow Cyt, WB, IHC-P, IP

**Species reactivity**  
Reacts with: Mouse, Rat, Human, African green monkey

**Immunogen**  
Synthetic peptide within Human AMPK alpha 1 aa 500 to the C-terminus (C terminal). The exact sequence is proprietary.  
Database link: Q13131

**Positive control**  
WB: HeLa, HepG2, C6, NIH/3T3 and MCF-7 cell lysate.  
IHC-P: Human cervical carcinoma and lung carcinoma tissues.  
ICC/IF: MCF-7 cells.  
Flow Cyt: HeLa cells.  
IP: HeLa cell lysate.

**General notes**

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

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

**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. Avoid freeze / thaw cycle.

**Storage buffer**  
pH: 7.20  
Preservative: 0.01% Sodium azide
Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

**Purity**
Protein A purified

**Clonality**
Monoclonal

**Clone number**
Y365

**Isotype**
IgG

**Function**
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.

**Sequence similarities**
Belongs to the protein kinase superfamily. CAMK Ser/Thr protein kinase family. SNF1 subfamily. Contains 1 protein kinase domain.

**Applications**

Our **Abpromise guarantee** covers the use of **ab32047** in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td></td>
<td>1/250.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td></td>
<td>1/100 - 1/150. &lt;br&gt;<strong>ab172730</strong> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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<tr>
<td>WB</td>
<td><strong>⭐⭐⭐⭐⭐</strong>&lt;br&gt;1/1000 - 1/5000. Predicted molecular weight: 63 kDa. This antibody shows low affinity on mouse and rat samples.</td>
<td></td>
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<tr>
<td>IHC-P</td>
<td></td>
<td>1/100 - 1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See <a href="#">IHC antigen retrieval protocols</a>. Mouse and rat species are recommended by WB application.</td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td>1/40 - 1/50.</td>
</tr>
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</table>

**Target**

**Function**
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.

**Sequence similarities**
Belongs to the protein kinase superfamily. CAMK Ser/Thr protein kinase family. SNF1 subfamily. Contains 1 protein kinase domain.

**Images**
**Lane 1**: Wild-type HAP1 cell lysate (20 µg)
**Lane 2**: AMPK alpha knockout HAP1 cell lysate (20 µg)
**Lane 3**: MCF7 cell lysate (20 µg)
**Lane 4**: HeLa knockout HAP1 cell lysate (20 µg)
**Lanes 1 - 4**: Merged signal (red and green). Green - ab32047 observed at 63 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab32047 was shown to specifically react with AMPK alpha in wild-type HAP1 cells. No band was observed when AMPK alpha knockout samples were examined. Wild-type and AMPK alpha knockout samples were subjected to SDS-PAGE. ab32047 and ab8245 (loading control to GAPDH) were diluted 1/5000 and 1/2000 respectively 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 1/10,000 dilution for 1hr at room temperature before imaging.

**Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections)** analysis of human lung carcinoma tissue labelling AMPK alpha 1 with purified ab32047 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.
Immunocytochemistry/Immunofluorescence analysis of MCF7 cells labelling AMPK alpha 1 with purified ab32047 at 1/250. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse anti-tubulin (1/1000) and ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500) were also used.

Control 1: primary antibody (1/250) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Control 2: ab7291 (1/1000) and secondary antibody, ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).

Flow Cytometry analysis of HeLa cells labelling AMPK alpha 1 with purified ab32047 at 1/150 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.
**Western blot - Anti-AMPK alpha 1 antibody [Y365] (ab32047)**

**All lanes**: Anti-AMPK alpha 1 antibody [Y365] (ab32047) at 1/2000 dilution (purified)

**Lane 1**: C6 cell lysate  
**Lane 2**: NIH/3T3 cell lysate

Lysates/proteins at 20 µg per lane.

**Secondary**  
**All lanes**: Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

**Predicted band size**: 63 kDa  
**Observed band size**: 63 kDa

Blocking and dilution buffer: 5% NFDM/TBST.  
This antibody shows low affinity on mouse and rat samples.

**Western blot - Anti-AMPK alpha 1 antibody [Y365] (ab32047)**

**All lanes**: Anti-AMPK alpha 1 antibody [Y365] (ab32047) at 1/10000 dilution (purified)

**Lane 1**: MCF7 cell lysate  
**Lane 2**: HepG2 cell lysate  
**Lane 3**: HeLa cell lysate  
**Lane 4**: K562 cell lysate

Lysates/proteins at 20 µg per lane.

**Secondary**  
**All lanes**: Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/10000 dilution

**Predicted band size**: 63 kDa  
**Observed band size**: 63 kDa

Blocking and dilution buffer: 5% NFDM/TBST.
ab32047 (purified) at 1/40 immunoprecipitating AMPK alpha 1 in HeLa whole cell lysate.

Lane 1 (input): HeLa whole cell lysate (10µg)
Lane 2 (+): ab32047 + HeLa whole cell lysate (10µg).
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab32047 in HeLa whole cell lysate.

For western blotting, ab131366 VeriBlot for IP (HRP) was used as the secondary antibody (1/1500).

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

All lanes: Anti-AMPK alpha 1 antibody [Y365] (ab32047) at 1/5000 dilution (unpurified)

Lane 1: MCF-7 cell lysate
Lane 2: HeLa cell lysate

Predicted band size: 63 kDa
Observed band size: 63 kDa
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue labelling AMPK alpha 1 with unpurified ab32047 at a dilution of 1/100.

Overlay histogram showing HeLa cells stained with unpurified ab32047 (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 (unpurified ab32047, 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 (monoclonal) (1μg/1x10⁶ cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

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