## Product datasheet

**Anti-GAPDH antibody [mAbcam 9484] - Loading Control ab9484**

★★★★★ 60 Abreviews  391 References  9 Images

### Overview

<table>
<thead>
<tr>
<th><strong>Product name</strong></th>
<th>Anti-GAPDH antibody [mAbcam 9484] - Loading Control</th>
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<tbody>
<tr>
<td><strong>Description</strong></td>
<td>Mouse monoclonal [mAbcam 9484] to GAPDH - Loading Control</td>
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<tr>
<td><strong>Host species</strong></td>
<td>Mouse</td>
</tr>
<tr>
<td><strong>Tested applications</strong></td>
<td>Suitable for: WB, IHC-P, Flow Cyt</td>
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<tr>
<td><strong>Species reactivity</strong></td>
<td>Reacts with: Mouse, Rat, Rabbit, Chicken, Cow, Dog, Human, Pig, Xenopus laevis, Cynomolgus monkey, Chinese hamster</td>
</tr>
<tr>
<td><strong>Immunogen</strong></td>
<td>Full length native protein (purified) corresponding to Human GAPDH.</td>
</tr>
<tr>
<td><strong>General notes</strong></td>
<td>For Western blotting, do not use milk for blocking. Our labs have extensively tested the blocking conditions for this antibody and recommend using 5% BSA for 1 hour. The comparison data are shown in the images section. This antibody clone [mAbcam 9484] is manufactured by Abcam. If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact <a href="mailto:orders@abcam.com">orders@abcam.com</a> or you can find further information here.</td>
</tr>
</tbody>
</table>

### Properties

<table>
<thead>
<tr>
<th><strong>Form</strong></th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Storage instructions</strong></td>
<td>Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.</td>
</tr>
</tbody>
</table>
| **Storage buffer** | pH: 7.4  
Preservative: 0.02% Sodium azide  
Constituents: PBS, 6.97% L-Arginine |
| **Purity**        | IgG fraction |
| **Clonality**     | Monoclonal |
| **Clone number**  | mAbcam 9484 |
| **Myeloma**       | Sp2/0-Ag14 |
| **Isotype**       | IgG2b |
| **Light chain type** | kappa |
Function

Has both glyceraldehyde-3-phosphate dehydrogenase and nitrosylase activities, thereby playing a role in glycolysis and nuclear functions, respectively. Participates in nuclear events including transcription, RNA transport, DNA replication and apoptosis. Nuclear functions are probably due to the nitrosylase activity that mediates cysteine S-nitrosylation of nuclear target proteins such as SIRT1, HDAC2 and PRKDC (By similarity). Glyceraldehyde-3-phosphate dehydrogenase is a key enzyme in glycolysis that catalyzes the first step of the pathway by converting D-glyceraldehyde 3-phosphate (G3P) into 3-phospho-D-glyceroyl phosphate.

Pathway

Carbohydrate degradation; glycolysis; pyruvate from D-glyceraldehyde 3-phosphate: step 1/5.

Sequence similarities

Belongs to the glyceraldehyde-3-phosphate dehydrogenase family.

Post-translational modifications

S-nitrosylation of Cys-152 leads to interaction with SIAH1, followed by translocation to the nucleus. ISGylated.

Cellular localization

Cytoplasm > cytosol. Nucleus. Cytoplasm > perinuclear region. Membrane. Translocates to the nucleus following S-nitrosylation and interaction with SIAH1, which contains a nuclear localization signal (By similarity). Postnuclear and Perinuclear regions.

Applications

Our Abpromise guarantee covers the use of ab9484 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>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>🟠🟠🟠🟠</td>
<td>Use a concentration of 0.1 - 1 µg/ml. Predicted molecular weight: 36 kDa. Do not block with milk. Block with 5% BSA for 1 hour. Our labs have thoroughly investigated the blocking conditions for this antibody. We found that milk significantly decreases the signal and is therefore not a suitable blocking agent for this antibody (see images).</td>
</tr>
<tr>
<td>IHC-P</td>
<td>🟠🟠🟠🟠</td>
<td>Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td>🟠🟠🟠🟠</td>
<td>Use 1µg for $10^6$ cells. ab170192 - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody. We recommend Goat Anti-Mouse IgG H&amp;L (DyLight® 488) preadsorbed (ab96879) secondary antibody</td>
</tr>
</tbody>
</table>

Target

Images
**Western blot - Anti-GAPDH antibody [mAbcam 9484] - Loading Control (ab9484)**

**All lanes**: Anti-GAPDH antibody [mAbcam 9484] - Loading Control (ab9484) at 1/5000 dilution

**Lane 1**: Hela whole cell (Human)
**Lane 2**: 3T3 cell (Mouse)
**Lane 3**: Rat brain
**Lane 4**: Xenopus embryo
**Lane 5**: Chicken Liver
**Lane 6**: EBTr cell (Cow)
**Lane 7**: CHO cell (Chinese hamster)
**Lane 8**: Pig liver

**Secondary**

**All lanes**: Rabbit Anti-Mouse IgG H&L (HRP) (ab6728) at 1/5000 dilution

Performed under reducing conditions.

**Predicted band size**: 36 kDa
**Observed band size**: 40 kDa

*why is the actual band size different from the predicted?*

**Exposure time**: 10 seconds

The membrane was blocked in 5% BSA in TBST for 1 hour, then incubated for 1 hour in primary antibody diluted in TBST.
Western blot - Anti-GAPDH antibody [mAbcam 9484] - Loading Control (ab9484) at 1/2000 dilution

**All lanes**
- Anti-GAPDH antibody [mAbcam 9484] - Loading Control (ab9484) at 1/2000 dilution

**Lane 1**: Chinese hamster ovary whole cell lysates
**Lane 2**: Bortezomib treated (100nM for 16 hours) Chinese hamster ovary whole cell lysates

**Secondary**
- All lanes: HRP-conjugated goat anti-mouse polyclonal IgG (H+L) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size**: 36 kDa
**Observed band size**: 41 kDa

*why is the actual band size different from the predicted?*

**Exposure time**: 1 minute

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Western blot - Anti-GAPDH antibody [mAbcam 9484] - Loading Control (ab9484) at 1/200 dilution

**All lanes**
- Anti-GAPDH antibody [mAbcam 9484] - Loading Control (ab9484) at 1/200 dilution

**Lane 1**: Rat intestinal epithelial (RIE-1) whole cell lysates
**Lane 2**: Bortezomib treated (100nM for 16 hours) rat intestinal epithelial (RIE-1) whole cell lysates

Lysates/proteins at 100000 cells per lane.

**Secondary**
- All lanes: Rat anti-rabbit IgG (H+L) IgG polyclonal at 1/5000 dilution

Developed using the ECL technique.
Predicted band size: 36 kDa

Observed band size: 41 kDa

why is the actual band size different from the predicted?

Exposure time: 1 minute

Lanes 1-5: Anti-GAPDH antibody [mAbcam 9484] - Loading Control (ab9484) at 1/1000 dilution (Blocked in 5% milk)

Lanes 6-10: Anti-GAPDH antibody [mAbcam 9484] - Loading Control (ab9484) at 1/1000 dilution (Blocked in 5% BSA)

Lanes 1 & 6: HeLa (Human epithelial carcinoma cell line) Nuclear Lysate
Lanes 2 & 7: HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lanes 3 & 8: A431 whole cell lysate (ab7909)
Lanes 4 & 9: Jurkat whole cell lysate (ab7899)
Lanes 5 & 10: HEK293 whole cell lysate (ab7902)

Lysates/proteins at 20 µg per lane.

Secondary
Lanes 1-5: Goat anti-Mouse (HRP conjugated) at 1/5000 dilution
Lanes 6-10: Goat anti-Mouse (HRP conjugated) at 1/5000 dilution

Predicted band size: 36 kDa

Observed band size: 40 kDa

why is the actual band size different from the predicted?

The membrane 1-5 was blocked in 5% milk (1 hour). The membrane 6-10 was blocked in 5% BSA (1 hour). Milk is not a suitable blocking agent and significantly decreases the signal on the membrane.
Western blot - Anti-GAPDH antibody [mAbcam 9484] - Loading Control (ab9484)

Anti-GAPDH antibody [mAbcam 9484] - Loading Control (ab9484) at 0.5 µg/ml + HeLa cell lysate

Secondary
Goat Anti-Mouse IgG H&L (HRP) (ab6789) at 1/5000 dilution

Developed using the ECL technique.

Performed under non-reducing conditions.

**Predicted band size:** 36 kDa

**Exposure time:** 30 seconds

IHC image of GAPDH staining in human liver FFPE section, performed on a Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab9484, 5µg/ml, for 8 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.
Immunohistochemical staining of human ovary cystadenocarcinoma with ab9484 at 1/200. Samples were incubated with the primary antibody for 14 hours at 4°C in PBS/5% goat serum. A HRP conjugated goat anti-mouse was used as the secondary at a dilution of 1/2000.

Overlay histogram showing HeLa cells stained with ab9484 (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 (ab9484, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was goat anti-mouse DyLight® 488 (IgG H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (ab91366, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

ab9484 staining GAPDH in A549 lung cancer cells by Flow Cytometry. Cells were harvested in trypsin, fixed with paraformaldehyde and permeabilized with 0.2% Triton X-100. The sample was incubated with the primary antibody (1/100 in 1x HBSS + 0.02% Triton X-100 + 1.5% FBS) for 3 hours at 25°C. An Alexa Fluor® 488-conjugated goat anti-mouse IgG polyclonal (1/2000) was used as the secondary antibody.

Gating Strategy: No gating.

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