

Product datasheet

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

★★★★★ 60 Abreviews 430 References 5 Images

Overview

Product name	Anti-GAPDH antibody [mAbcam 9484] - Loading Control
Description	Mouse monoclonal [mAbcam 9484] to GAPDH - Loading Control
Host species	Mouse
Tested applications	Suitable for: WB, IHC-P, Flow Cyt
Species reactivity	Reacts with: Mouse, Rat, Rabbit, Chicken, Cow, Dog, Human, Pig, Xenopus laevis, Cynomolgus monkey, Chinese hamster
Immunogen	Full length native protein (purified) corresponding to Human GAPDH.
General notes	<p>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.</p> <p>This antibody clone [mAbcam 9484] is manufactured by Abcam.</p> <p>If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information here.</p> <p>Abcam recommended secondaries - Goat Anti-Mouse HRP (ab205719) and Goat Anti-Mouse Alexa Fluor® 488 (ab150113).</p> <p>See other anti-mouse secondary antibodies that can be used with this antibody.</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 or -80°C. Avoid freeze / thaw cycle.
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

Applications

Our [Abpromise guarantee](#) covers the use of **ab9484** in the following tested applications.

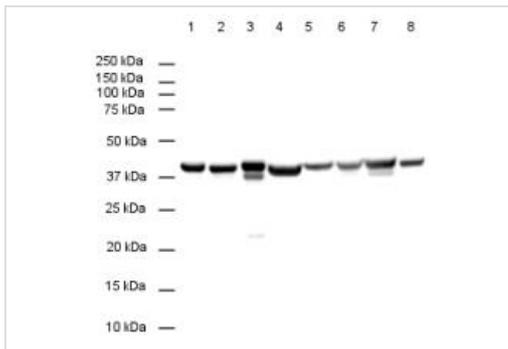
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★	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).
IHC-P	★★★★★	Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
Flow Cyt	★★★★☆	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&L (DyLight® 488) preadsorbed (ab96879) secondary antibody

Target

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.

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.

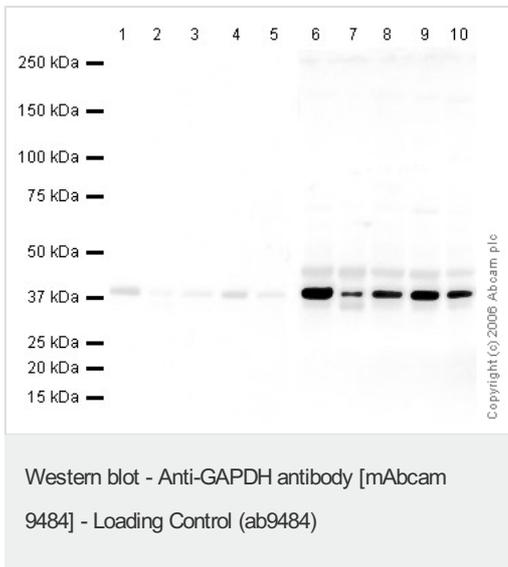
Predicted band size: 36 kDa

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.



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.



Anti-GAPDH antibody [mAbcam 9484] - Loading Control (ab9484)

at 0.5 µg/ml + HeLa cell lysate

Secondary

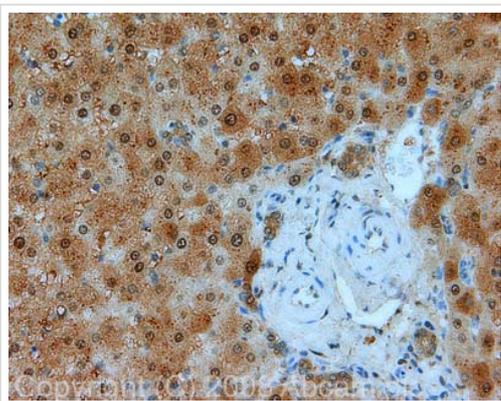
Goat Anti-Mouse IgG H&L (HRP) ([ab6789](#)) at 1/5000 dilution

Developed using the ECL technique.

Performed under non-reducing conditions.

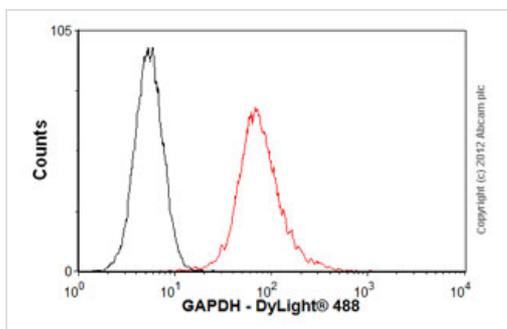
Predicted band size: 36 kDa

Exposure time: 30 seconds



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GAPDH antibody [mAbcam 9484] - Loading Control (ab9484)

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.



Flow Cytometry - Anti-GAPDH antibody [mAbcam 9484] - Loading Control (ab9484)

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/1x10⁶ 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/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed.

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