abcam

Product datasheet

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

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Overview

Product name	Anti-GAPDH antibody [mAbcam 9484] - Loading Control
Description	Mouse monoclonal [mAbcam 9484] to GAPDH - Loading Control
Host species	Mouse
Specificity	In western blot, this product typically gives a lower signal in rat lysates compared to human and mouse lysates.
Tested applications	**Suitable for:** WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Chicken, Cow, Human, Pig, Xenopus laevis, Chinese hamster **Predicted to work with:** Rabbit, Dog, Cynomolgus monkey
Immunogen	Full length native protein (purified). This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: A431, Jurkat, HEK293, HeLa, NIH 3T3, PC-12, EBTr and CHO cell lysates; Rat brain, chicken and pig liver and Xenopus laevis embryo tissue lysates. IHC-P: Human liver tissue.
General notes	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 is 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 **orders@abcam.com** or you can find further information **here**. 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. 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

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: 7.40 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

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab9484 in the following tested applications.

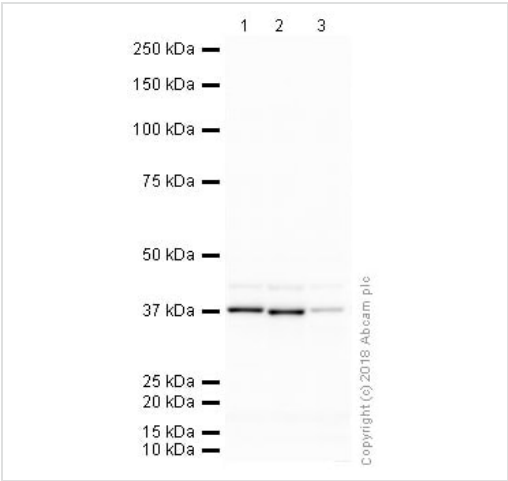
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★ **(53)**	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.

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 µg/ml

Lane 1 : HeLa Whole Cell Lysate
Lane 2 : NIH 3T3 Whole Cell Lysate
Lane 3 : PC12 Whole Cell Lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/5000 dilution

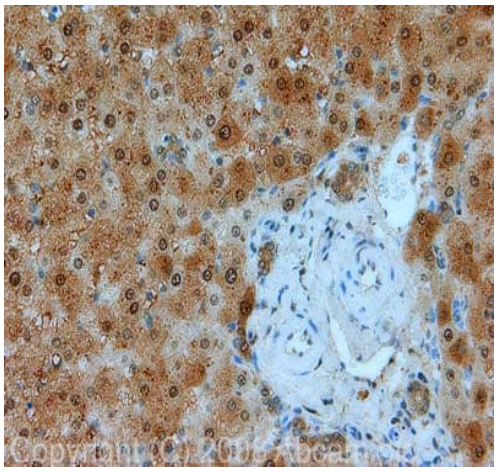
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 36 kDa

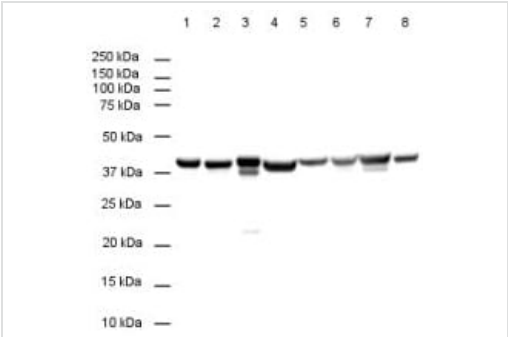
Exposure time: 1 minute

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab9484 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution **ab133406**



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.



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 laevis embryo
Lane 5 : Chicken Liver
Lane 6 : EBTr cell (Cow)
Lane 7 : CHO cell (Chinese hamster)
Lane 8 : Pig liver

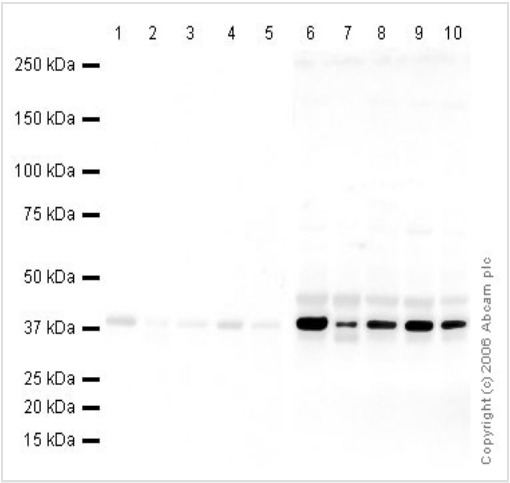
Secondary
All lanes : Rabbit Anti-Mouse IgG H&L (HRP) (**ab6728**) at 1/5000 dilution

Performed under reducing conditions.

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

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)

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)

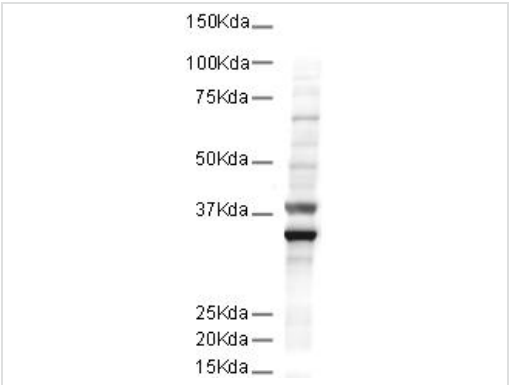
Lanes 1 & 6 : HeLa (Human epithelial carcinoma cell line) Nuclear Lysate
Lanes 2 & 7 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lanes 3 & 8 : A-431 whole cell lysate (**ab7909**)
Lanes 4 & 9 : Jurkat whole cell lysate (**ab7899**)
Lanes 5 & 10 : HEK-293 whole cell lysate (**ab7902**)

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

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

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