


# Anti-GAPDH antibody [6C5] - Loading Control ab8245

★★★★★ [100 Abreviews](#) [3490 References](#) [6 Images](#)

### Overview

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<b>Product name</b>	Anti-GAPDH antibody [6C5] - Loading Control
<b>Description</b>	Mouse monoclonal [6C5] to GAPDH - Loading Control
<b>Host species</b>	Mouse
<b>Specificity</b>	This GAPDH antibody can be used as a loading control antibody. GAPDH is a 146 kDa tetramer composed of four 30-40 kDa subunits. There is no cross-reaction with GAPDH from yeast. Preliminary data indicates that the GAPDH antibody- loading control ab8245 recognizes the monomer (36 kDa) and also the dimer forms of GAPDH, but not the tetrameric form of the protein.
<b>Tested applications</b>	<b>Suitable for:</b> WB, ICC/IF
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human <b>Predicted to work with:</b> Horse, Chicken, Guinea pig, Hamster, Cat, Dog, Pig, Xenopus laevis, Fish, Monkey, Zebrafish, Baboon, Xenopus tropicalis  <b>Does not react with:</b> Goat, Cow, Saccharomyces cerevisiae
<b>Immunogen</b>	Full length native protein (purified) corresponding to GAPDH. Database link: <a href="#">P46406</a>
<b>Positive control</b>	ICC/IF: HeLa cells, NIH3T3 cells, SV40LT-SMC cells. WB: HeLa, A431, Jurkat, HEK-293, Raji whole cell lysate.
<b>General notes</b>	<p>This product switched from ascites to tissue culture supernatant on 31 July 2017. Lot numbers higher than [GR291713] will be from tissue culture supernatant.</p> <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&amp;As</p>

### Properties

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<b>Form</b>	Liquid
<b>Storage instructions</b>	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.

<b>Storage buffer</b>	pH: 7.40 Preservative: 0.09% Sodium azide Constituent: PBS
<b>Purity</b>	Protein A purified
<b>Purification notes</b>	Chromatography on protein A Sepharose
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	6C5
<b>Myeloma</b>	Sp2/0
<b>Isotype</b>	IgG1

## Applications

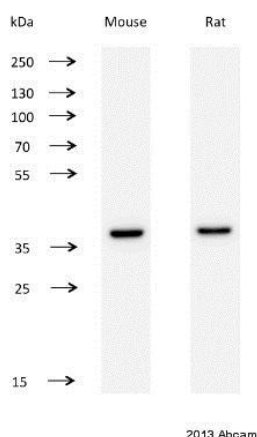
**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab8245 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
<b>WB</b>	★★★★★ (96)	1/500 - 1/10000. Detects a band of approximately 36 kDa (predicted molecular weight: 40.2 kDa).
<b>ICC/IF</b>	★★★★★ (1)	Use a concentration of 1 - 5 µg/ml.

## Target

<b>Function</b>	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.
<b>Pathway</b>	Carbohydrate degradation; glycolysis; pyruvate from D-glyceraldehyde 3-phosphate: step 1/5.
<b>Sequence similarities</b>	Belongs to the glyceraldehyde-3-phosphate dehydrogenase family.
<b>Post-translational modifications</b>	S-nitrosylation of Cys-152 leads to interaction with SIAH1, followed by translocation to the nucleus. ISGylated.
<b>Cellular localization</b>	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 [6C5] - Loading Control (ab8245)

This image is courtesy of an anonymous Abreview

**All lanes :** Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

**Lane 1 :** Mouse hippocampus whole cell lysate

**Lane 2 :** Rat hippocampus whole cell lysate

Lysates/proteins at 20 µg per lane.

### Secondary

**All lanes :** HRP-conjugated Rabbit anti-mouse at 1/5000 dilution

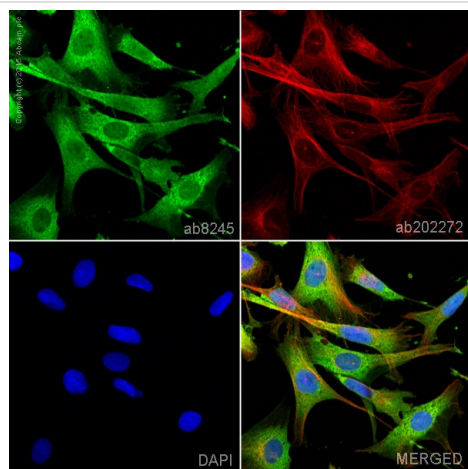
Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size:** 40.2 kDa

**Observed band size:** 36 kDa

**Exposure time:** 10 seconds

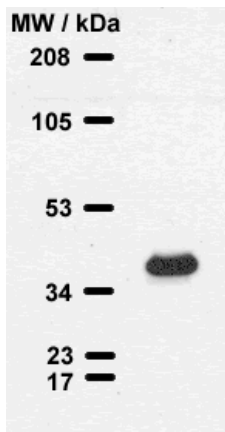


Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

ab8245 staining GAPDH in SV40LT-SMC (Rat SV40-transfected aorta smooth cell line) cells.

The cells were fixed with 4% formaldehyde (10 minutes), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1 hour. The cells were then incubated with ab8245 at 5µg/ml and **ab202272** at 1/250 overnight at +4°C, followed by a further incubation at room temperature for 1h with Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed (**ab150117**) (shown in green). Nuclear DNA was labeled in blue with DAPI.

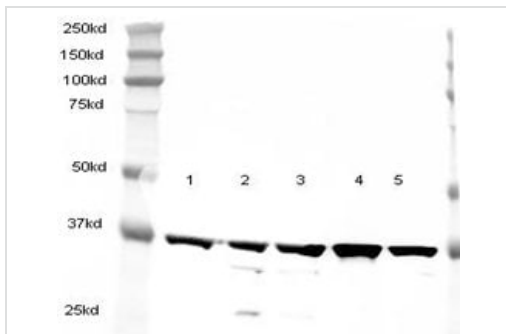
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

Anti-GAPDH antibody [6C5] - Loading Control (ab8245) at 10  $\mu\text{g/ml}$  + Raji (Human Burkitt's lymphoma cell line) whole cell lysate at 20  $\mu\text{g}$

**Predicted band size:** 40.2 kDa



Western blot - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

**All lanes :** Anti-GAPDH antibody [6C5] - Loading Control (ab8245) at 2.5  $\mu\text{g/ml}$

**Lane 1 :** HeLa (Human epithelial cell line from cervix adenocarcinoma) Nuclear

**Lane 2 :** HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

**Lane 3 :** A431 (Human epidermoid carcinoma cell line) cell lysate

**Lane 4 :** Jurkat (Human T cell leukemia cell line from peripheral blood) cell lysate

**Lane 5 :** HEK-293 (Human epithelial cell line from embryonic kidney) cell lysate

Lysates/proteins at 20  $\mu\text{g}$  per lane.

### Secondary

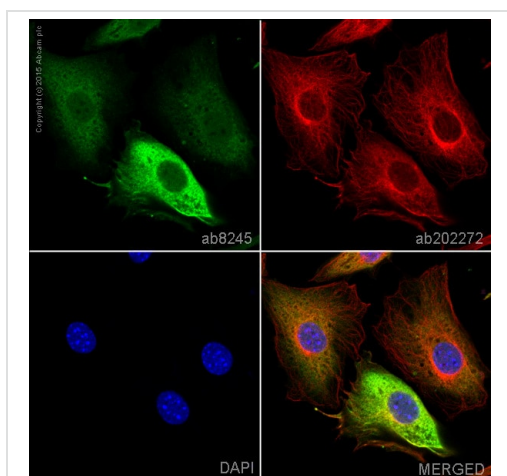
**All lanes :** Alexa Fluor anti-mouse at 1/5000 dilution

Performed under reducing conditions.

**Predicted band size:** 40.2 kDa

**Observed band size:** 37 kDa

Fluorescence detection of secondary antibody.

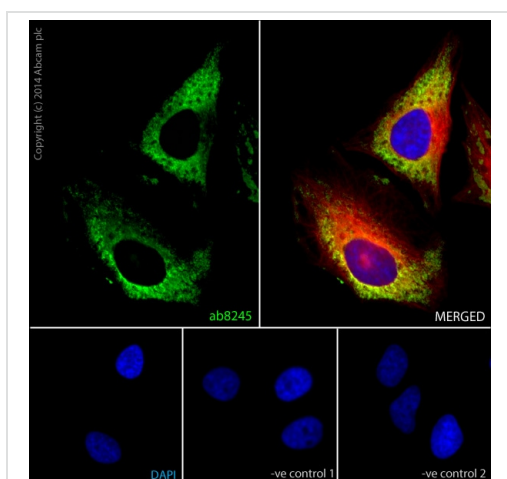


Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

ab8245 staining GAPDH in NIH/3T3 (Mouse embryo fibroblast cell line) cells.

The cells were fixed with 4% formaldehyde (10 minutes) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1 hour. The cells were then incubated with ab8245 at 1 µg/ml and [ab202272](#) at 1/250 overnight at +4°C, followed by a further incubation at room temperature for 1 hour with Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed ([ab150117](#)) (shown in green). Nuclear DNA was labeled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [6C5] - Loading Control (ab8245)

ab8245 staining GAPDH in HeLa (Human epithelial cell line from cervix adenocarcinoma) cells.

The cells were fixed with 100% methanol (5 minutes) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1 hour. The cells were then incubated with ab8245 at 5 µg/ml and [ab6046](#) at 1 µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1 hour with Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed ([ab150117](#)) at 2 µg/ml (shown in green) and Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed ([ab150088](#)) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labeled in blue with DAPI.

**Negative controls:** 1– Rabbit primary antibody and anti-mouse secondary antibody; 2 – Mouse primary antibody and anti-rabbit secondary antibody. Controls 1 and 2 indicate that there is no unspecific reaction between primary and secondary antibodies used.

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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