**Anti-GAPDH antibody [6C5] ab8245**

**Overview**

**Product name** Anti-GAPDH antibody [6C5]

**Description** Mouse monoclonal [6C5] to GAPDH

**Host species** Mouse

**Specificity** This antibody can be used as a loading control antibody, however we also recommend ab9484 as an alternative. 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 antibody recognizes the monomer (36 kDa) and also the dimer forms of GAPDH, but not the tetrameric form of the protein.

**Tested applications** Suitable for: ELISA, ICC, WB, ICC/IF, IHC-Fr

**Species reactivity** Reacts with: Mouse, Rat, Rabbit, Chicken, Hamster, Cat, Dog, Human, Pig, Xenopus laevis, Fish, Monkey, Zebrafish, Baboon, African green monkey

Predicted to work with: Horse, Guinea pig, Xenopus tropicalis

**Immunogen** Rabbit muscle GAPDH.

**Positive control** ICC/IF: HeLa cells, NIH3T3 cells, SV40LT-SMC cells

**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: 0.09% Sodium azide
Constituent: PBS

**Purity** Protein A purified

**Purification notes** Chromatography on protein A Sepharose

**Clonality** Monoclonal

**Clone number** 6C5

**Myeloma** Sp2/0

**Isotype** IgG1
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

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<td>ELISA</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>ICC</td>
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<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/500 - 1/10000. Detects a band of approximately 36 kDa (predicted molecular weight: 40.2 kDa). PubMed: 16450009</td>
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<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 1 - 5 µg/ml.</td>
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<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/500.</td>
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Target

Images
All lanes: Anti-GAPDH antibody [6C5] (ab8245) at 2.5 µg/ml

Lane 1: HeLa Nuclear
Lane 2: HeLa whole cell lysate
Lane 3: A431 cell lysate
Lane 4: Jurkat cell lysate
Lane 5: HEK293 cell lysate

Lysates/proteins at 20 µg 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.

ab8245 staining GAPDH in SV40LT-SMC cells. The cells were fixed with 4% formaldehyde (10min), 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 1h. 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 labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).
**Western blot - Anti-GAPDH [6C5] antibody (ab8245)**

This image is courtesy of an anonymous Abreview.

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

ab8245 staining GAPDH in NIH3T3 cells. The cells were fixed with 4% formaldehyde (10min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab8245 at 1µg/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 labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).
ab8245 staining GAPDH in HeLa cells. The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab8245 at 5μg/ml and ab6046 at 1μg/ml 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) 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 labelled 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.
All lanes: Anti-GAPDH antibody [6C5] (ab8245) at 1/20000 dilution

Lane 1: Rabbit aorta, whole tissue lysate at 10 µg
Lane 2: Blank
Lane 3: Mouse aorta, whole tissue lysate at 5 µg
Lane 4: Mouse aorta, whole tissue lysate at 7.5 µg
Lane 5: Mouse aorta, whole tissue lysate at 10 µg
Lane 6: Mouse aorta, whole tissue lysate at 12.5 µg
Lane 7: Mouse aorta, whole tissue lysate at 15 µg

Secondary
All lanes: HRP conjugated sheep anti-mouse antibody at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 40.2 kDa

Exposure time: 30 seconds

20 µg of Raji whole cell lysate (ab7908) probed with mouse monoclonal to GAPDH (ab8245) at 10µg/ml.

20 µg of Raji whole cell lysate (ab7908) probed with mouse monoclonal to GAPDH (ab8245) at 10µg/ml.
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