Overview

Product name: Anti-GAPDH antibody [6C5] - Loading Control

Description: Mouse monoclonal [6C5] to GAPDH - Loading Control

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 ▲ Does not react with: Goat, Cow, Saccharomyces cerevisiae

Immunogen: Rabbit muscle GAPDH.

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

General notes: Abcam recommended secondaries - Goat Anti-Mouse HRP (ab205719) and Goat Anti-Mouse Alexa Fluor® 488 (ab150113).

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

Applications

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.

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<th>Abreviews</th>
<th>Notes</th>
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<tr>
<td>ELISA</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>ICC</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<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>
</tr>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 1 - 5 µg/ml.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/500.</td>
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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
<table>
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<tr>
<th>Lane</th>
<th>Sample Description</th>
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<tr>
<td>1</td>
<td>HeLa (Human epithelial cell line from cervix adenocarcinoma) Nuclear</td>
</tr>
<tr>
<td>2</td>
<td>HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate</td>
</tr>
<tr>
<td>3</td>
<td>A431 (Human epidermoid carcinoma cell line) cell lysate</td>
</tr>
<tr>
<td>4</td>
<td>Jurkat (Human T cell leukemia cell line from peripheral blood) cell lysate</td>
</tr>
<tr>
<td>5</td>
<td>HEK-293 (Human epithelial cell line from embryonic kidney) cell lysate</td>
</tr>
</tbody>
</table>

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

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

Fluorescence detection of secondary antibody.
ab8245 staining GAPDH in SV40LT-SMC 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).

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

**Exposure time**: 10 seconds
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).

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

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

Anti-GAPDH antibody [6C5] - Loading Control (ab8245) at 10 µg/ml + Raji (Human Burkitt's lymphoma cell line) whole cell lysate at 20 µg

**Predicted band size**: 40.2 kDa

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