Anti-GAPDH antibody [EPR6256] - Loading Control

Overview

Product name: Anti-GAPDH antibody [EPR6256] - Loading Control

Description: Rabbit monoclonal [EPR6256] to GAPDH - Loading Control

Host species: Rabbit

Tested applications: Suitable for: WB, IP, Flow Cyt, ICC/IF, IHC-P

Species reactivity: Reacts with: Human, African green monkey

Does not react with: Mouse

Immunogen: Synthetic peptide within Human GAPDH aa 250 to the C-terminus. The exact sequence is proprietary.

Database link: P04406


General notes: Our RabMab® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents

This product is a recombinant rabbit monoclonal antibody.

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. Stable for 12 months at -20°C.

Storage buffer: pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: 9% PBS, 40% Glycerol, 0.05% BSA, 50% Tissue culture supernatant

Purity: Tissue culture supernatant

Clonality: Monoclonal

Clone number: EPR6256

Isotype: IgG
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
Our Abpromise guarantee covers the use of ab128915 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>★★★★★</td>
<td>1/10000 - 1/50000. Detects a band of approximately 35 kDa (predicted molecular weight: 36 kDa).</td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td>1/10 - 1/100.</td>
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<tr>
<td>Flow Cyt</td>
<td></td>
<td>1/100 - 1/1000. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>★★★★★</td>
<td>1/250 - 1/500. 2.0 µg/ml</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>1/250.</td>
</tr>
</tbody>
</table>

Target

Images
**Western blot - Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915)**

**All lanes**: Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915) at 1/10000 dilution

**Lane 1**: HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cell lysate

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

**Lane 3**: HepG2 (human liver hepatocellular carcinoma cell line) cell lysate

**Lane 4**: HUVEC (human umbilical vein endothelial cell line) cell lysate

**Lane 5**: MCF7 (human breast adenocarcinoma cell line) cell lysate

**Lane 6**: SH-SY5Y (human neuroblastoma cell line from bone marrow) cell lysate

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes**: HRP labelled Goat anti-Rabbit IgG at 1/2000 dilution

**Predicted band size**: 36 kDa

**Observed band size**: 35 kDa

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

Secondary antibody - **anti-rabbit HRP (ab6721)**
IHC image of ab128915 staining GAPDH in human pancreas* formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. 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 ab128915, 1:250 dilution, for 15 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. No primary antibody was used in the secondary only control (shown on the inset).

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

ab128915 staining GAPDH in HeLa (human epithelial cell line from cervix adenocarcinoma) 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 ab128915 at 2μg/ml and ab7291 at 1μg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with an goat anti-rabbit AlexaFluor®488 (ab150081) at 2 μg/ml (shown in green) and goat anti-mouse AlexaFluor®594 (ab150120) 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.
Overlay histogram showing HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained with ab128915 (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 (ab128915, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was goat anti-rabbit Alexa Fluor® 488 (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1μg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

ab128915 staining GAPDH in human HeLa (human epithelial cell line from cervix adenocarcinoma) cells by ICC/IF (Immunocytochemistry/imunofluorescence). Cells were fixed with paraformaldehyde and permeabilized with 0.5% Triton X-100 in PBS. Samples were incubated with primary antibody (1/500 in PBS) for 1 hour at 22°C. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG polyclonal (1/200) was used as the secondary antibody. Counter stained with DAPI.

ab128915, at 1/250, staining GAPDH in MCF7 (human breast adenocarcinoma cell line) cells by immunofluorescence.

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