# abcam

## Product datasheet

## Anti-GAPDH antibody [EPR16891] - Loading Control ab181602

Recombinant RabMAb

\*\*\*\* 22 Abreviews 2087 References 12 Images

### Overview

**Product name** Anti-GAPDH antibody [EPR16891] - Loading Control

**Description** Rabbit monoclonal [EPR16891] to GAPDH - Loading Control

**Host species** Rabbit

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

Species reactivity Reacts with: Mouse, Rat, Chicken, Human, Zebrafish, African green monkey, Xenopus tropicalis

Predicted to work with: Rabbit, Fish

Recombinant fragment. This information is proprietary to Abcam and/or its suppliers. **Immunogen** 

Positive control WB: HeLa, UMNSAH/DF-1, Jurkat, COS-1, RAW 264.7 and PC-12 whole cell lysates; Human

> fetal brain and heart lysates; Xenopus(X. tropicalis) muscle lysate; Zebrafish lysate; Mouse kidney and spleen lysates; Rat brain lysate. IHC-P: Human transitional cell carcinoma of bladder, Mouse spleen and Rat spleen tissues. ICC/IF: HeLa cells. Flow: Jurkat cells. IP: HeLa whole cell extract

**General notes** This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

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

#### **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 long

term. Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

**Purity** Protein A purified

Clone number Monoclonal EPR16891

**Isotype** IgG

#### **Applications**

## The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab181602 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
Flow Cyt (Intra)		1/180. <b>ab172730</b> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB	**** (19)	1/10000. Detects a band of approximately 36 kDa (predicted molecular weight: 36 kDa).
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF	*** <u>*</u>	1/500.
IP		1/60.

## **Target**

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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.

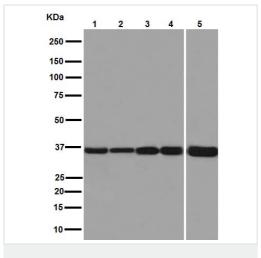
ISGylated.

**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 [EPR16891] - Loading Control (ab181602)

**All lanes :** Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) at 1/10000 dilution

Lane 1: Mouse kidney lysates

Lane 2: Mouse spleen lysates

Lane 3: RAW 264.7 (Mouse macrophage cells transformed with

Abelson murine leukemia virus) whole cell lysates

Lane 4: PC-12 (Rat adrenal gland pheochromocytoma) whole cell

lysates

Lane 5: Rat brain lysates

Lysates/proteins at 10 µg per lane.

## **Secondary**

**All lanes :** Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

**Predicted band size:** 36 kDa **Observed band size:** 36 kDa

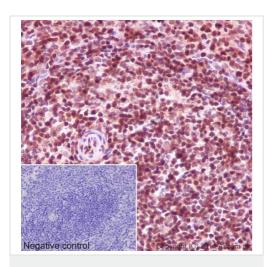
Blocking/Dilution buffer: 5% NFDM/TBST.

ab181602 MERGED

PAPI —-ye control 1 —-ye control 2

Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602)

Immunocytochemistry/immunofluorescence staining of 4% paraformaldehyde fixed; 0.1% triton X 100 permeabilized HeLa (human cervix adenocarcinoma) cells labeling GAPDH with ab181602 at dilution of 1/500. The secondary antibody used was Alexa Fluor<sup>®</sup> 488; goat anti-rabbit lgG (ab150077) at a dilution of 1/400. Nucleus was counter-stained with DAPI (blue). ab7291, a mouse anti-tubulin antibody (1/500) was used to stain tubulin along with ab150120 (AlexaFluor<sup>®</sup>594 goat anti-mouse secondary, 1/500). The negative controls are shown in the bottom middle and right hand panels- for negative control 1 primary antibody (ab181602; 1/500) and secondary antibody (ab150120; 1/500) and secondary antibody (ab7291; 1/500) and secondary antibody (ab150077; 1/400) was used.



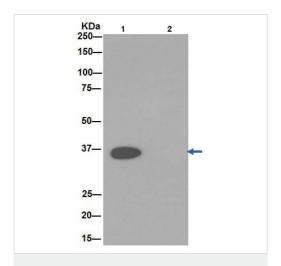
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GAPDH antibody

[EPR16891] - Loading Control (ab181602)

Immunohistochemical analysis of paraffin-embedded rat spleen tissue labeling GAPDH with ab181602 at 1/2000 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Nucleus and cytoplasmic staining on lymphocyte of rat spleen is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

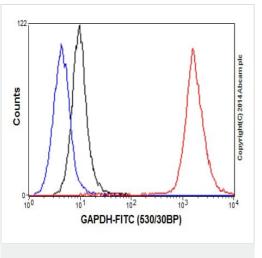


Immunoprecipitation - Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602)

GAPDH was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell extract with ab181602 at 1/60 dilution. Western blot was performed from the immunoprecipitate using ab181602 at 1/5000 dilution. Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG, was used as secondary antibody at 1/1500 dilution. **Lane 1:** HeLa whole cell extract.

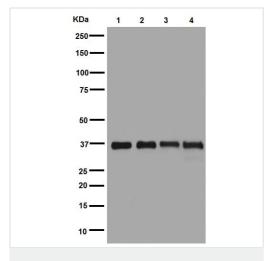
Lane 2: PBS instead of HeLa whole cell extract.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602)

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed Jurkat (Human T cell leukemia cells from peripheral blood) cells labeling GAPDH with ab181602 at 1/180 dilution (red) compared with a rabbit monoclonal lgG isotype control (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit lgG (FITC) at 1/150 dilution was used as the secondary antibody.



Western blot - Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602)

**All lanes :** Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) at 1/50000 dilution

**Lane 1 :** HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysates

Lane 2: Xenopus tropicalis muscle lysates

Lane 3: UMNSAH/DF-1 (Transformed chicken embyronic fibroblast cells) whole cell lysates

Lane 4 : Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysates

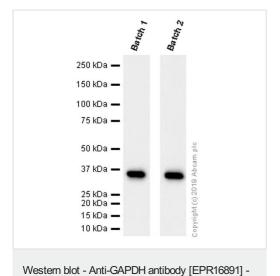
Lysates/proteins at 20 µg per lane.

## Secondary

**All lanes :** Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

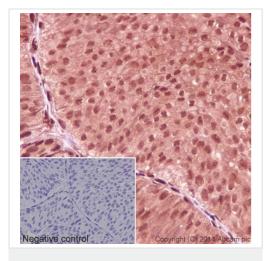
Predicted band size: 36 kDa
Observed band size: 36 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



Loading Control (ab181602)

Different batches of ab181602 were tested on HeLa (Human cervix adenocarcinoma epithelial cell) lysate at 1.0  $\mu$ g/ml. 15  $\mu$ g of lysate was loaded in each lane. Bands observed at 36 kDa.

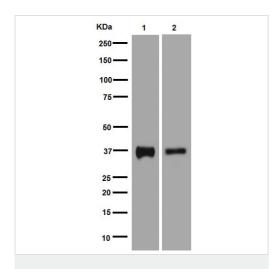


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GAPDH antibody
[EPR16891] - Loading Control (ab181602)

Immunohistochemical analysis of paraffin-embedded human transitional cell carcinoma of bladder tissue labeling GAPDH with ab181602 at 1/2000 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Cytoplasmic and nucleus staining on the tumor cells of transitional cell carcinoma of human bladder is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-GAPDH antibody [EPR16891] -Loading Control (ab181602)

All lanes: Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) at 1/10000 dilution

Lane 1: COS-1 (African green monkey kidney fibroblast-like cell line) whole cell lysates

Lane 2: Zebrafish lysates

Lysates/proteins at 20 µg per lane.

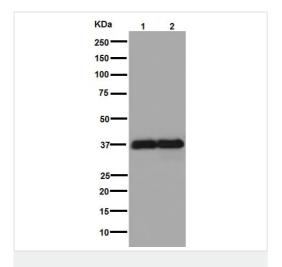
## Secondary

All lanes: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 36 kDa

Observed band size: 36 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-GAPDH antibody [EPR16891] -Loading Control (ab181602)

All lanes: Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) at 1/10000 dilution

Lane 1: Human fetal brain lysates Lane 2: Human fetal heart lysates

Lysates/proteins at 10 µg per lane.

## Secondary

All lanes: Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 36 kDa Observed band size: 36 kDa Blocking/Dilution buffer: 5% NFDM/TBST.

Negative control

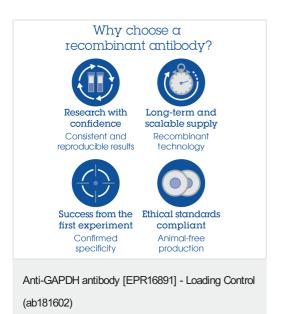
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[EPR16891] - Loading Control (ab181602)

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling GAPDH with ab181602 at 1/2000 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Nucleus and cytoplasmic staining on lymphocytes of mouse spleen is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



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