abcam

Product datasheet

Anti-GAPDH antibody [EPR16891] - Loading Control ab181602

Recombinant RabMAb

16 Abreviews 269 References 10 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:** WB, IHC-P, ICC/IF, Flow Cyt, IP
Species reactivity	**Reacts with:** Mouse, Rat, Rabbit, Chicken, Human, Fish, Monkey, Zebrafish, Xenopus tropicalis
Immunogen	Recombinant fragment within Mouse GAPDH aa 100 to the C-terminus. The exact sequence is proprietary. Database link: P16858 Run BLAST with Run BLAST with
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	Abcam recommended secondaries - Goat Anti-Rabbit HRP (ab205718) and Goat Anti-Rabbit Alexa Fluor® 488 (ab150077). See other anti-rabbit secondary antibodies that can be used with this antibody. 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 long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR16891
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab181602** in the following tested applications.

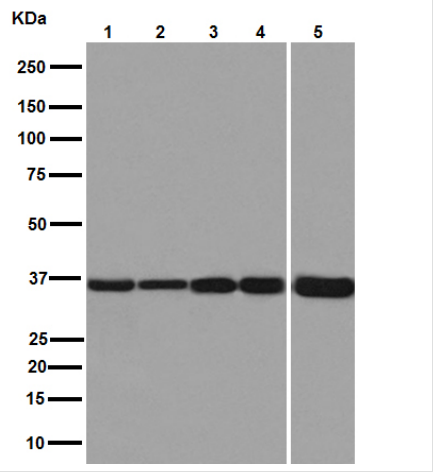
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★	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	★★★★★	1/500.
Flow Cyt		1/180. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP		1/60.

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



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

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

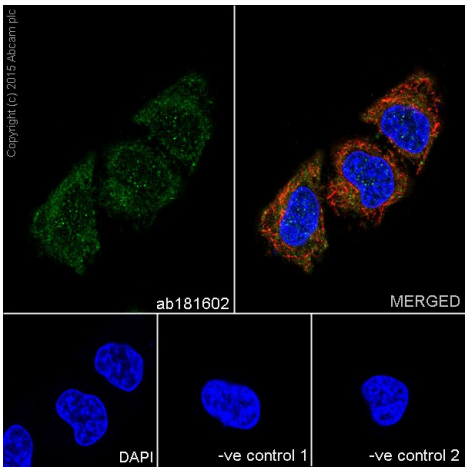
Lane 1 : Mouse kidney lysates
Lane 2 : Mouse spleen lysates
Lane 3 : RAW 264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) whole cell lysates
Lane 4 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysates
Lane 5 : Rat brain lysates

Lysates/proteins at 10 µg per lane.

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

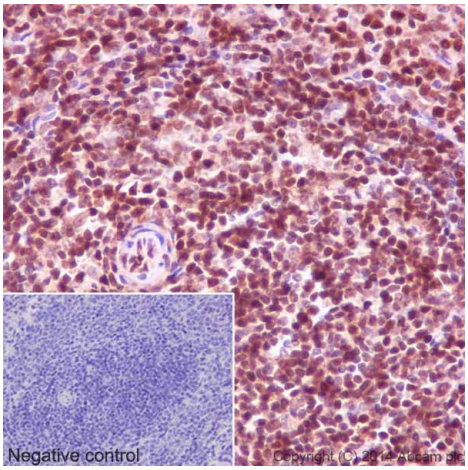
Predicted band size: 36 kDa
Observed band size: 36 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



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

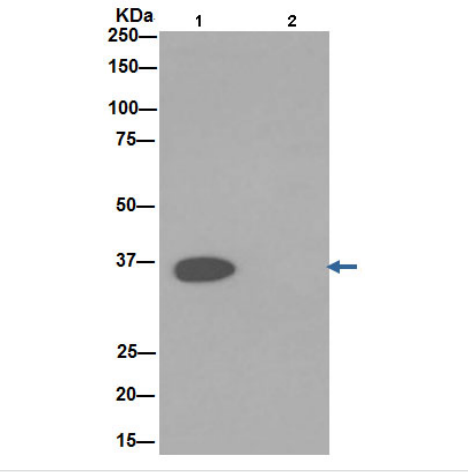
Immunocytochemistry/immunofluorescence staining of 4% paraformaldehyde fixed; 0.1% triton X 100 permeabilized HeLa (human cervix adenocarcinoma) cells labeling GAPDH with ab181602 at dilution of 1/500. The secondary antibody used was Alexa Fluor® 488; goat anti-rabbit IgG (ab150077) at a dilution of 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®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) was used. For negative control 2 primary antibody (ab7291; 1/500) and secondary antibody (ab150077; 1/400) was used.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded 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.

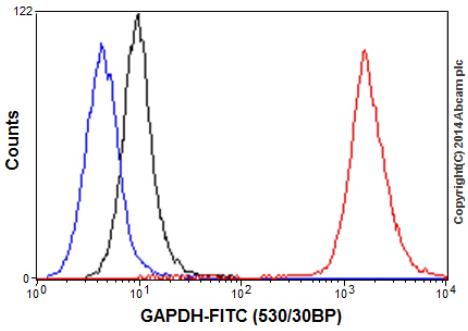


Immunoprecipitation - Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602)

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 IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution. **Lane 1:** HeLa whole cell extract.

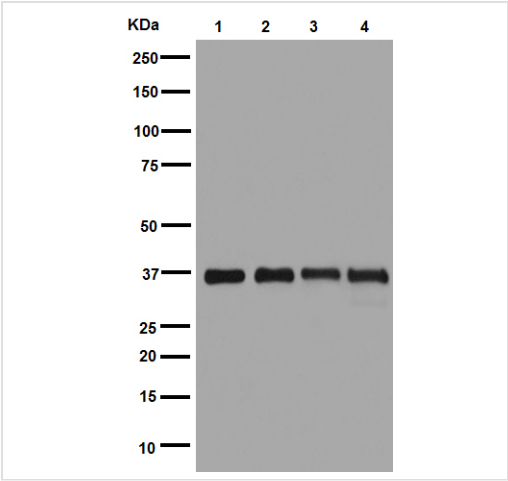
Lane 2: PBS instead of HeLa whole cell extract.

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



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

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 IgG isotype control (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (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

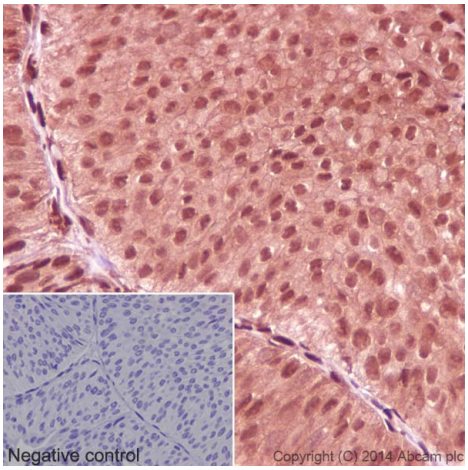
Lane 1 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysates
Lane 2 : Xenopus(X. tropicalis) muscle lysates
Lane 3 : UMNSAH/DF-1 (Transformed chicken embyronic fibroblast cells) whole cell lysates
Lane 4 : Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysates

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

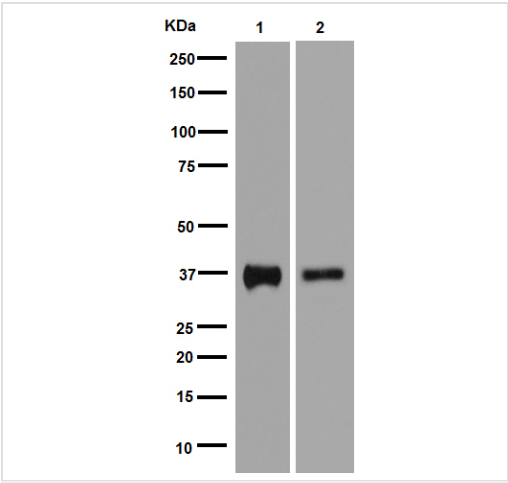
Blocking/Dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded 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.



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

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

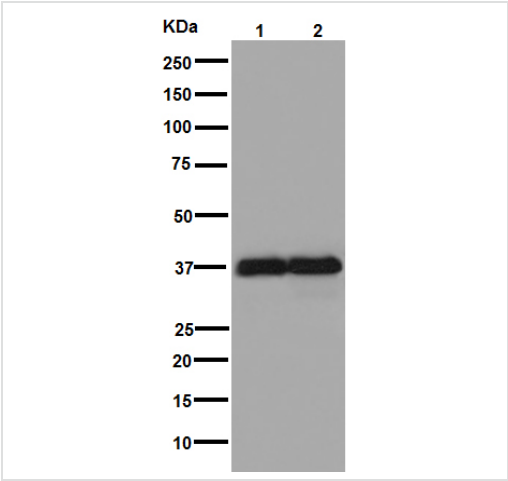
Lane 1 : COS-1 (African green monkey kidney fibroblast-like cell line) whole cell lysates
Lane 2 : Zebrafish lysates

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

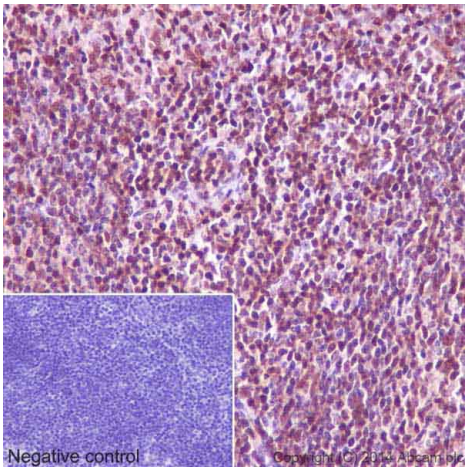
Lane 1 : Human fetal brain lysates
Lane 2 : Human fetal heart lysates

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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GAPDH antibody [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.

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