abcam

Product datasheet

Anti-GAPDH antibody [EPR16891] - BSA and Azide free ab199553

Recombinant RabMAb

★★★★★ 1 Abreviews 3 References 12 Images

Overview

Product name	Anti-GAPDH antibody [EPR16891] - BSA and Azide free
Description	Rabbit monoclonal [EPR16891] to GAPDH - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), IP, IHC-P, WB, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Chicken, Human, Zebrafish, African green monkey, Xenopus tropicalis **Predicted to work with:** Rabbit, Fish
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
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	ab199553 is the carrier-free version of **ab181602**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR16891
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab199553 in the following tested applications.

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

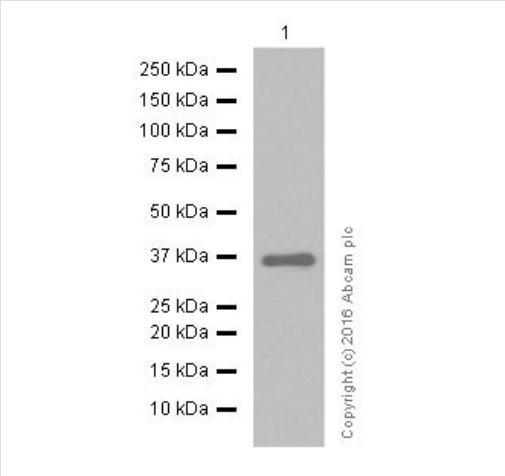
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★★★★★ **(1)**	Use at an assay dependent concentration. Detects a band of approximately 36 kDa (predicted molecular weight: 36 kDa).
ICC/IF		Use at an assay dependent concentration.

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] - BSA and Azide free (ab199553)

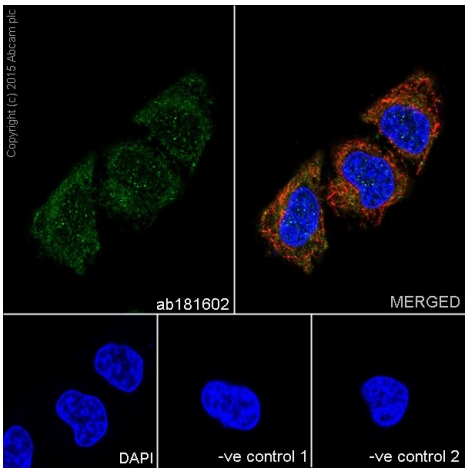
Anti-GAPDH antibody [EPR16891] - BSA and Azide free (ab199553) at 1/100000 dilution + HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysates at 15 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 36 kDa

Blocking and Diluting buffer and concentration:5% NFDM/TBST

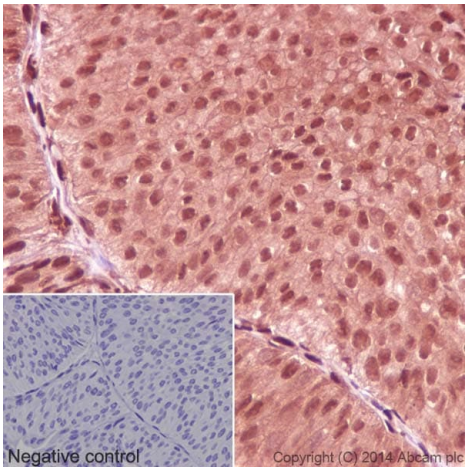
Exposure time:3 seconds



Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [EPR16891] - BSA and Azide free (ab199553)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181602**).



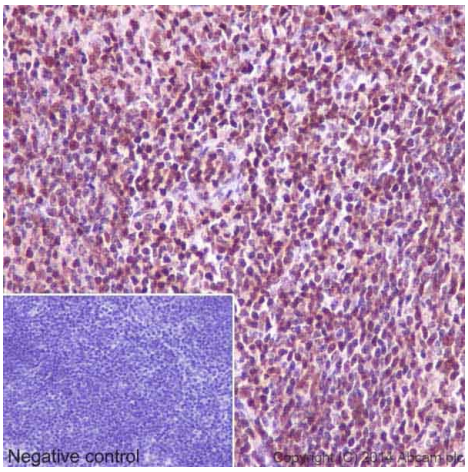
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GAPDH antibody [EPR16891] - BSA and Azide free (ab199553)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181602**).

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



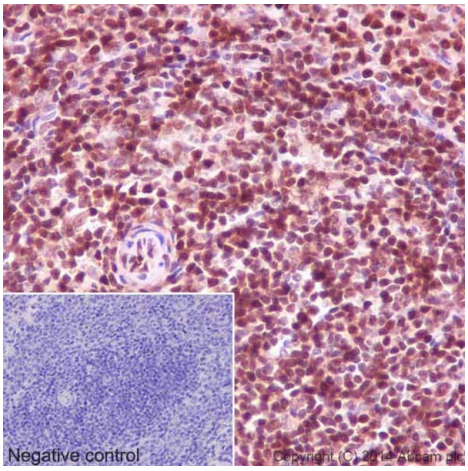
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GAPDH antibody [EPR16891] - BSA and Azide free (ab199553)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181602**).

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



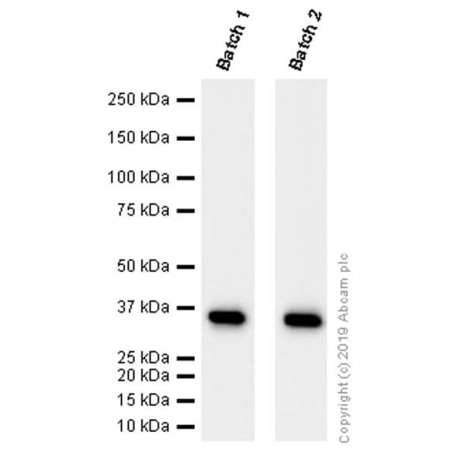
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GAPDH antibody [EPR16891] - BSA and Azide free (ab199553)

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.

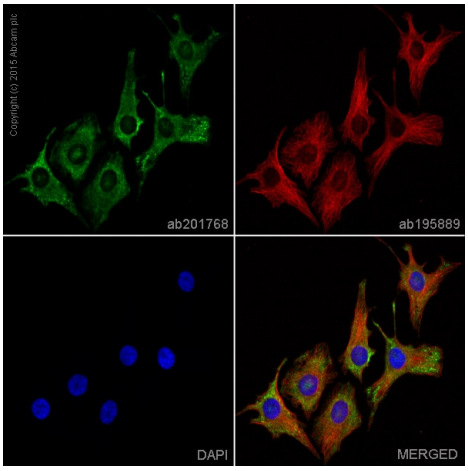
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181602**).

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



Western blot - Anti-GAPDH antibody [EPR16891] - BSA and Azide free (ab199553)

This data was developed using **ab181602**, the same antibody clone in a different buffer formulation. Different batches of **ab181602** were tested on HeLa (Human cervix adenocarcinoma epithelial cell) lysate at 1.0 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 36 kDa.

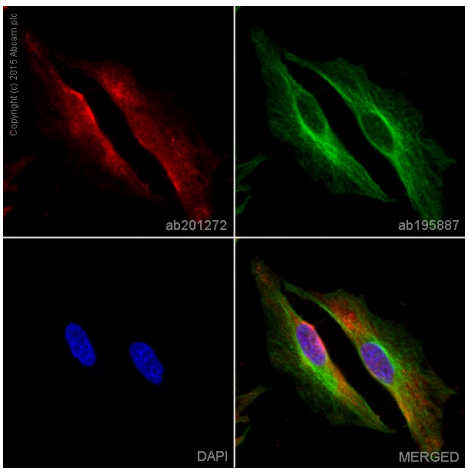


Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [EPR16891] - BSA and Azide free (ab199553)

Clone EPR16891 (ab199553) has been successfully conjugated by Abcam. This image was generated using Anti-GAPDH antibody [EPR16891] (Alexa Fluor® 488). Please refer to **ab201768** for protocol details.

ab201768 staining GAPDH in HeLa cells. The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab201768** at 1/200 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 2µg/ml (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

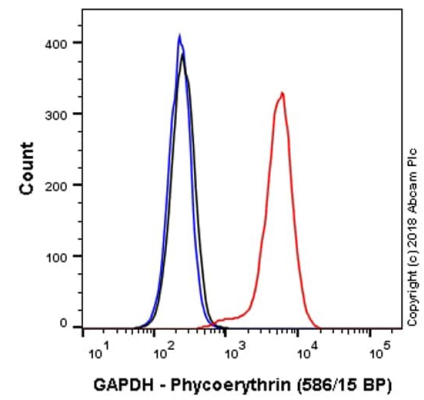


Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [EPR16891] - BSA and Azide free (ab199553)

Clone EPR16891 (ab199553) has been successfully conjugated by Abcam. This image was generated using Anti-GAPDH antibody [EPR16891] (Alexa Fluor® 647). Please refer to **ab201272** for protocol details.

ab201272 staining GAPDH in HeLa cells. The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab201272** at 1/100 dilution (shown in red) and **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 2µg/ml (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Flow Cytometry (Intracellular) - Anti-GAPDH antibody [EPR16891] - BSA and Azide free (ab199553)

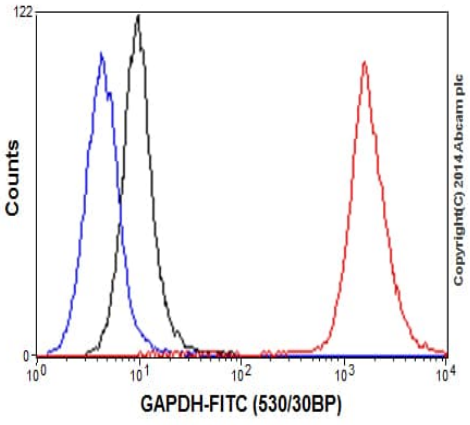
Clone EPR16891 (ab199553) has been successfully conjugated by Abcam. This image was generated using Anti-GAPDH antibody [EPR16891] (PE). Please refer to **ab224004** for protocol details.

Overlay histogram showing HeLa cells stained with **ab224004** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (**ab224004**, 1/1000 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was Rabbit IgG (monoclonal) Phycoerythrin (**ab209478**) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 50 mW Yellow/Green laser (561nm) and 586/15 bandpass filter.

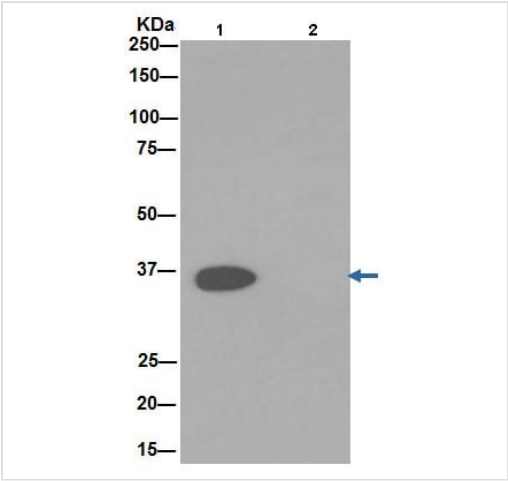
This antibody gave a positive signal in HeLa cells fixed with 4% formaldehyde (10 min)/permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.



Flow Cytometry (Intracellular) - Anti-GAPDH antibody [EPR16891] - BSA and Azide free (ab199553)

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 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181602**).



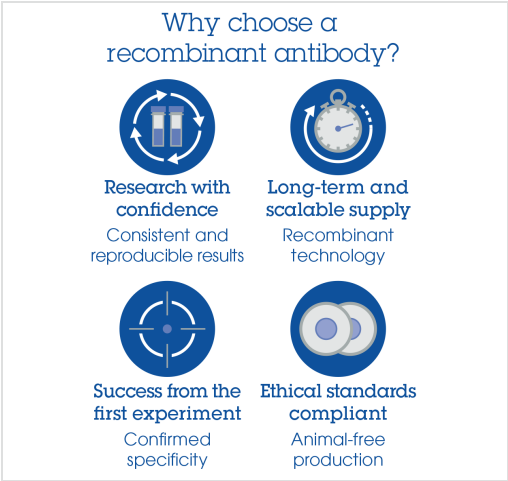
Immunoprecipitation - Anti-GAPDH antibody [EPR16891] - BSA and Azide free (ab199553)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181602**).



Anti-GAPDH antibody [EPR16891] - BSA and Azide free (ab199553)

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