abcam

ab186930

Anti-GAPDH antibody [EPR6256] - BSA and Azide free

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR6256
Purification technique	Affinity purification Protein A
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Human
Dilution info	-

Notes	-

Expected

Species	African green monkey
Dilution info	Use at an assay dependent concentration.
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	African green monkey
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	African green monkey
Dilution info	-
Notes	Please check the parent abID, ab128915, for a recommended dilution.

Species	Human
Dilution info	-
Notes	Please check the parent abID, ab128915, for a recommended dilution.

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	African green monkey
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	African green monkey
Dilution info	-
Notes	-

Target data

See full target information GAPDH

Function	Catalyzes the conversion of D-glyceraldehyde 3-phosphate (G3P) into 3-phospho-D-glyceroyl phosphate in glycolysis and the reverse reaction in gluconeogenesis (PubMed:11724794, PubMed:3170585). Also shows nitrosylase activity, thereby playing a role in nuclear functions (PubMed:11724794, PubMed:3170585). Modulates the organization and assembly of the cytoskeleton (By similarity). Facilitates the CHP1-dependent microtubule and membrane associations through its ability to stimulate the binding of CHP1 to microtubules (By similarity). Component of the GAIT (gamma interferon-activated inhibitor of translation) complex which mediates interferon-gamma-induced transcript-selective translation inhibition in inflammation processes (PubMed:23071094). Upon interferon-gamma treatment assembles into the GAIT complex which binds to

stem loop-containing GAIT elements in the 3'-UTR of diverse inflammatory mRNAs (such as ceruplasmin) and suppresses their translation (PubMed:23071094). Also plays a role in innate immunity by promoting TNF-induced NF-kappa-B activation and type I interferon production, via interaction with TRAF2 and TRAF3, respectively (PubMed:23332158, PubMed:27387501). Participates in nuclear events including transcription, RNA transport, DNA replication and apoptosis (By similarity). 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).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab186930 is the carrier-free version of ab128915.

Species reactivity
Mouse: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

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

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Glyceraldehyde-3-phosphate dehydrogenase commonly known as GAPDH is an enzyme involved in glycolysis. Its molecular weight (MW) is approximately 36 kDa. The protein is expressed ubiquitously in almost all tissues reflecting its essential role in energy production. GAPDH catalyzes the sixth step of glycolysis converting glyceraldehyde-3-phosphate into 13-bisphosphoglycerate. Due to its stable expression researchers often use GAPDH as a loading control in western blot experiments.
Biological function summary	GAPDH serves important metabolic functions beyond its enzymatic role in glycolysis. It functions as part of a multi-enzyme complex within the cytoplasm which facilitates efficient substrate channeling during glycolysis. Additionally GAPDH has non-glycolytic roles including involvement in nuclear processes like RNA export and DNA repair. Its ubiquitous presence across different cellular compartments indicates its multiple functions beyond metabolic pathways.
Pathways	GAPDH integrates into significant cellular functions like the glycolytic pathway and apoptotic pathways. In glycolysis GAPDH collaborates with enzymes like phosphoglycerate kinase forming a cohesive link in the energy conversion chain. Its participation in apoptotic pathways highlights GAPDH's involvement in cellular death processes interacting with proteins like Bcl-2 to influence apoptosis progression. These roles reinforce its presence in central metabolic and regulatory pathways.
Associated diseases and disorders	GAPDH has associations with neurodegenerative diseases and cancer. In neurodegenerative disorders such as Alzheimer's disease GAPDH’s altered enzymatic activity is frequently observed influencing cellular energy homeostasis. Moreover overexpression or aberrant regulation of GAPDH relates to cancer cell proliferation and metastasis implicating proteins like p53 in these pathways. The diverse functions and interactions of GAPDH emphasize its importance in both normal cellular function and disease states.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

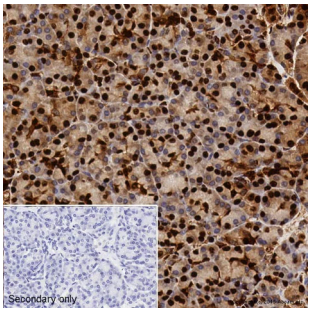
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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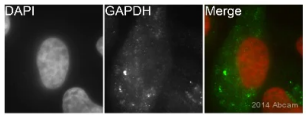
12 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930)

GAPDH Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-GAPDH antibody

IHC image of ab128915 (unpurified) 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
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab128915).



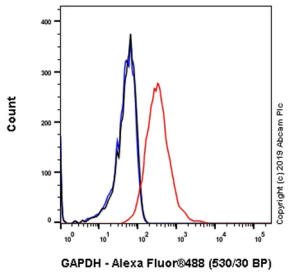
This image is courtesy of a customer review

Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930)

ab128915 (unpurified) staining GAPDH in human HeLa (human epithelial cell line from cervix adenocarcinoma) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde and permeabilized with 0.5% Triton X-100 in PBS. Samples were incubated

submitted by Kirk McManus

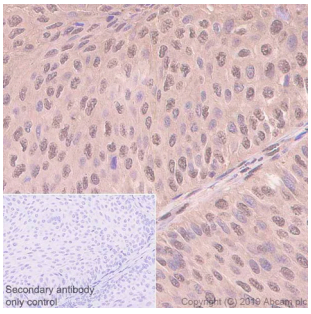
with primary antibody (1/500 in PBS) for 1 hour at 22°C. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG polyclonal (1/200) was used as the secondary antibody. Counter stained with DAPI. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab128915).



Flow Cytometry (Intracellular) - Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930)

GAPDH Flow Cytometry (Intracellular) staining using rabbit Anti-GAPDH antibody

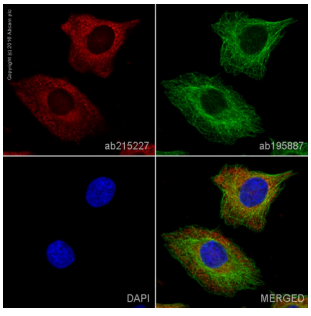
Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling GAPDH with purified ab128915 at 1/20 dilution (10µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue). This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab128915).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930)

GAPDH Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-GAPDH antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human bladder carcinoma tissue sections labeling GAPDH with purified ab128915 at 1/2000 dilution (0.06 µg/ml). Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab128915).

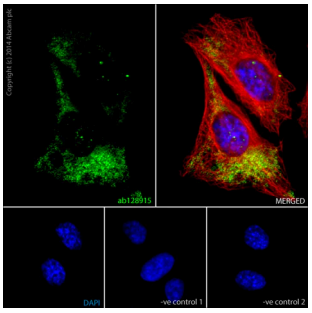


Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930)

GAPDH Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-GAPDH antibody

Clone EPR6256 (ab186930) has been successfully conjugated by Abcam. This image was generated using Anti-GAPDH antibody [EPR6256] (Alexa Fluor® 647). Please refer to ab215227 for protocol details.
ab215227 staining GAPDH in HeLa cells. The cells were fixed with 100% methanol (5 min), 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 ab215227 at 1/1000 dilution (shown in red) and ab195887, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

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

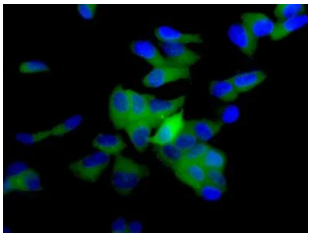


Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930)

GAPDH Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-GAPDH antibody

ab128915 (unpurified) 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.

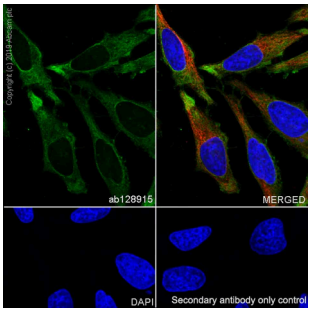
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab128915).



Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930)

GAPDH Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-GAPDH antibody

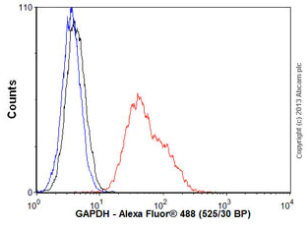
ab128915 (unpurified), at 1/250, staining GAPDH in MCF7 (human breast adenocarcinoma cell line) cells by immunofluorescence.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab128915).



Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930)

GAPDH Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-GAPDH antibody

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling GAPDH with purified ab128915 at 1/250 dilution (0.4 µg/ml). Cells were fixed in 100% Methanol. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 (2.5 µg/ml) dilution. Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab128915).

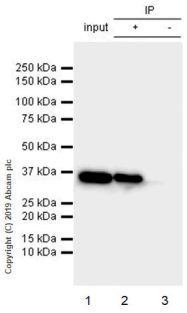


Flow Cytometry (Intracellular) - Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930)

GAPDH Flow Cytometry (Intracellular) staining using rabbit Anti-GAPDH antibody

Overlay histogram showing HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained with ab128915 (unpurified) (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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab128915).

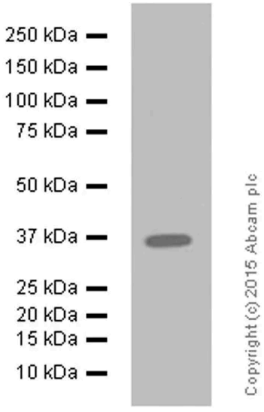


Immunoprecipitation - Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930)

ab128915 (purified) at 1/20 dilution (0.5ug) immunoprecipitating GAPDH in HeLa whole cell lysates.
Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates 10ug
Lane 2 (+): ab128915 & HeLa whole cell lysates
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab128915 in HeLa whole cell lysates
For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/1000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab128915).

All lanes:
Immunoprecipitation - Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915)

Predicted band size: 25 kDa, 36 kDa



Western blot - Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930)

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

All lanes:
Western blot - Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930)

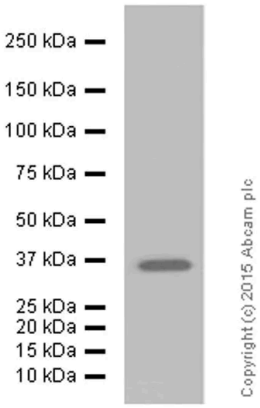
All lanes:
HEK293 (human embryonic kidney) whole cell lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051)

Predicted band size: 36 kDa

Exposure time: 30s



Western blot - Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930)

GAPDH Western blot staining of HeLa (human cervix adenocarcinoma) whole cell lysate using rabbit Anti-GAPDH antibody

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

All lanes:
Western blot - Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930)

All lanes:
HeLa (human cervix adenocarcinoma) whole cell lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051)

Predicted band size: 36 kDa

Exposure time: 1min

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.