abcam

Product datasheet

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

Recombinant RabMAb

5 References 13 Images

Overview

Product name	Anti-GAPDH antibody [EPR6256] - BSA and Azide free
Description	Rabbit monoclonal [EPR6256] to GAPDH - BSA and Azide free
Host species	Rabbit
Specificity	The African green monkey recommendation is based on the WB results. We do not guarantee IHC-P for African green monkey.
Tested applications	**Suitable for:** WB, ICC/IF, Flow Cyt (Intra), IP, IHC-P
Species reactivity	**Reacts with:** Human, African green monkey
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: 293T, HeLa, HepG2, HUVEC, MCF7 or SH SY5Y cell lysates. ICC/IF: MCF7 and HeLa cells IHC-P: Human bladder carcinomaFlow Cyt (intra): HeLa cells IP: HeLa cell lysate
General notes	ab186930 is the carrier-free version of **ab128915**. 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**.

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

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR6256
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab186930 in the following tested applications.

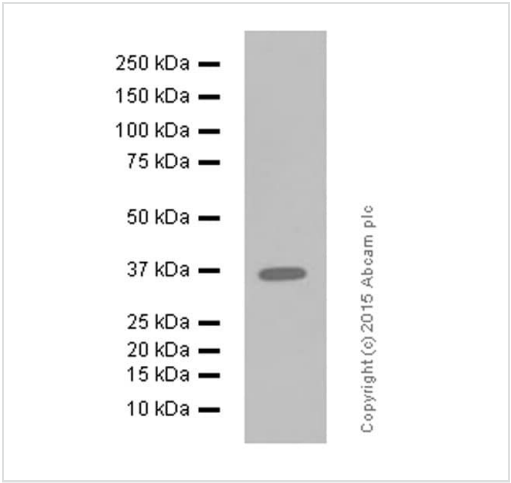
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 36 kDa. Please check the parent abID, **ab128915**, for a recommended dilution.
ICC/IF		Use at an assay dependent concentration.
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. See **IHC antigen retrieval protocols**. The African green monkey recommendation is based on the WB results. We do not guarantee IHC-P for African green monkey.

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 [EPR6256] - BSA and Azide free (ab186930)

Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930) (unpurified) + HEK293 (human embryonic kidney) whole cell lysate at 10 µg

Secondary

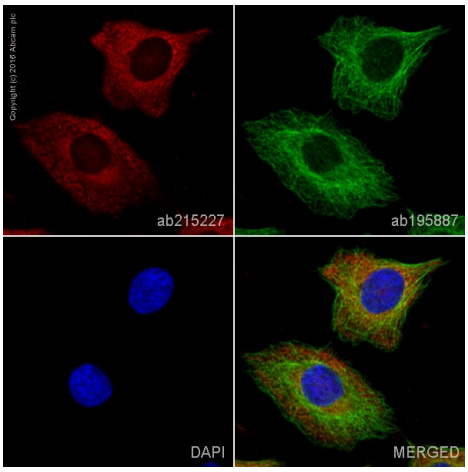
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**)

Predicted band size: 36 kDa

Exposure time: 30 seconds

Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM/TBST

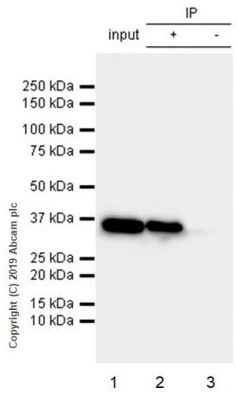


Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930)

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).

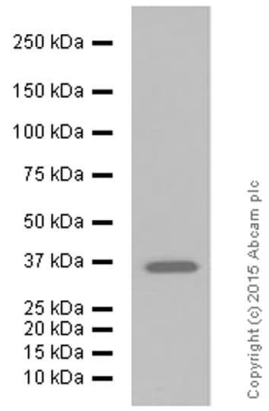
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



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**).



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

Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930) (unpurified) + HeLa (human cervix adenocarcinoma) whole cell lysate at 10 µg

Secondary

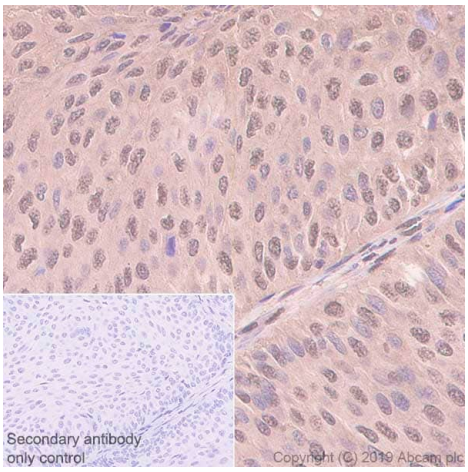
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**)

Predicted band size: 36 kDa

Exposure time: 1 minute

Blocking buffer and concentration: 5% NFDM/TBST

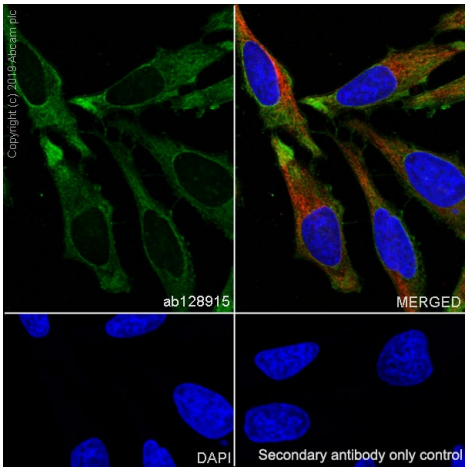
Diluting buffer and concentration: 5% NFDM/TBST



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

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.

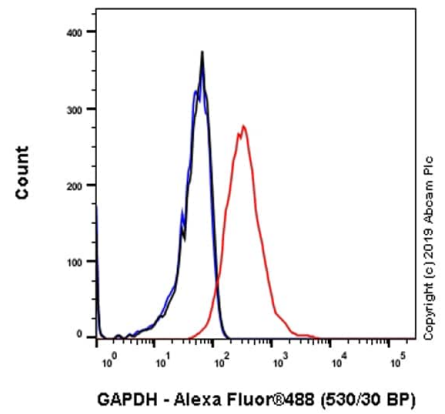
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab128915**).



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

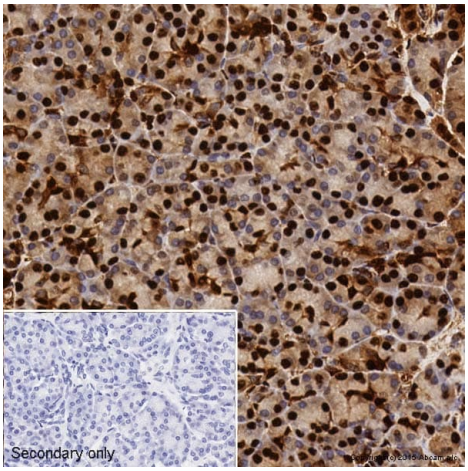
Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling GAPDH with purified **ab128915** at 1/250 dilution (0.4 µg/ml). Cells were fixed in 100% Methanol. Cells were counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule 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)

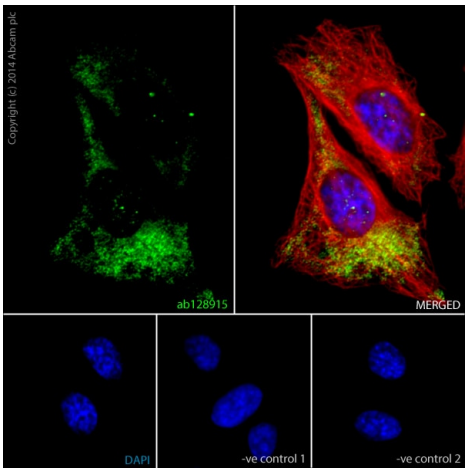
Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling GAPDH with purified **ab128915** at 1/20 dilution (10µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) 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)

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**).

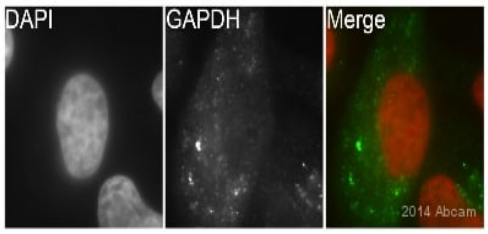


Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930)

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**).

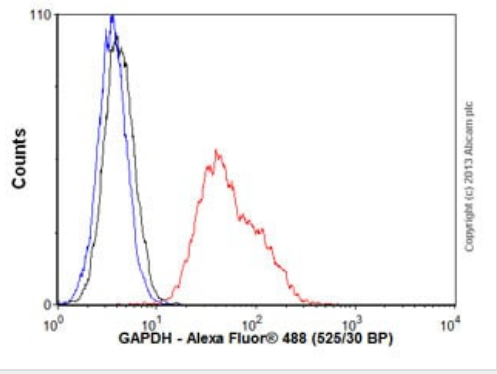


Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [EPR6256] - BSA and Azide free (ab186930)

This image is courtesy of an Abreview submitted by Kirk McManus.

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 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.

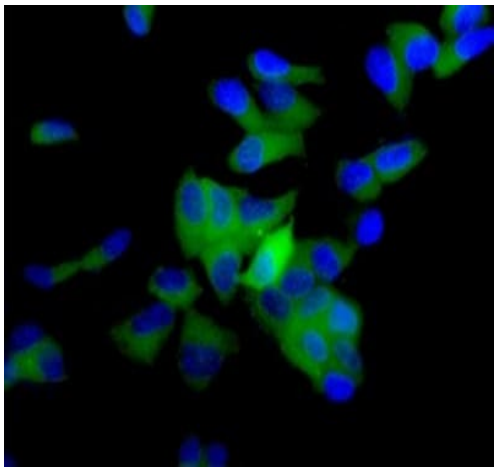
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab128915**).



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

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.

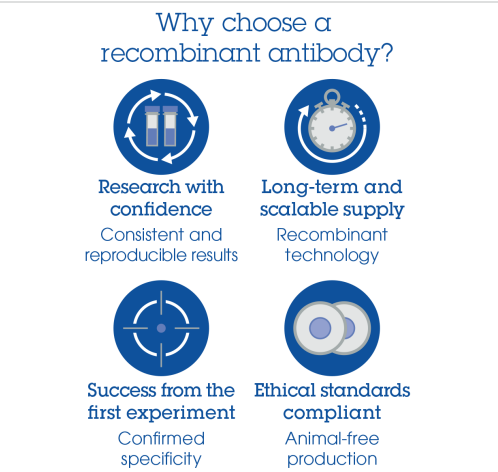
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab128915**).



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

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**).



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

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