abcam

Product datasheet

Anti-GAPDH antibody [EPR6256] - Loading Control ab128915

Recombinant RabMAb

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Overview

Product name	Anti-GAPDH antibody [EPR6256] - Loading Control
Description	Rabbit monoclonal [EPR6256] to GAPDH - Loading Control
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, IP, ICC/IF, IHC-P, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Human, African green monkey
Immunogen	Synthetic peptide within Human GAPDH aa 250 to the C-terminus. The exact sequence is proprietary. Database link: **P04406**
Positive control	WB: 293T, HeLa, HepG2, HUVEC, MCF7 and SH-SY5Y cell lysates. ICC/IF: HeLa and MCF7 cells. IHC-P: Human pancreas tissue, Human bladder carcinoma. Flow Cyt (intra): HeLa cells. IP: HeLa cell lysate.
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**. 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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

	Avoid freeze / thaw cycle. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR6256
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab128915 in the following tested applications.

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

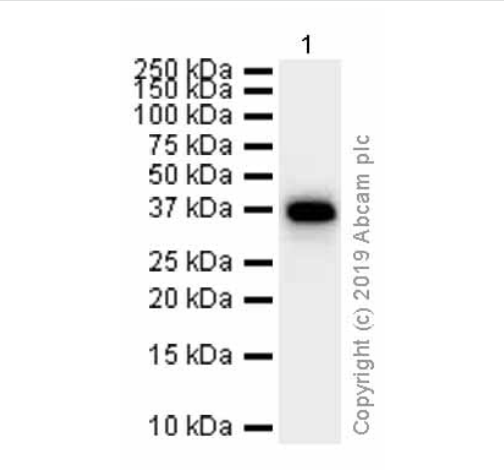
Application	Abreviews	Notes
WB	★★★★★ **(9)**	1/10000 - 1/50000. Detects a band of approximately 35 kDa (predicted molecular weight: 36 kDa).
IP		1/10 - 1/100.
ICC/IF	★★★★☆ **(1)**	1/250 - 1/500. 2.0 µg/ml
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. **For unpurified use at 1/250.** The African green monkey recommendation is based on the WB results. We do not guarantee IHC-P for African green monkey.
Flow Cyt (Intra)		1/20. For unpurified use at 1/100 - 1/1000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

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	S-nitrosylation of Cys-152 leads to interaction with SIAH1, followed by translocation to the

modifications	nucleus. lSGylated.
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] - Loading Control (ab128915)

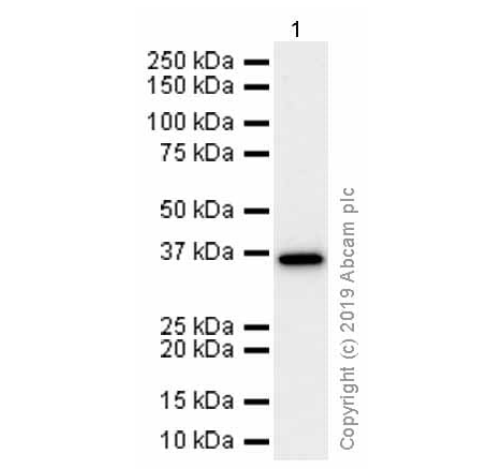
Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915) at 1/20000 dilution (Purified) + COS-1 (African green monkey kidney fibroblast-like) whole cell lysates at 15 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 36 kDa

Observed band size: 36 kDa



Western blot - Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915)

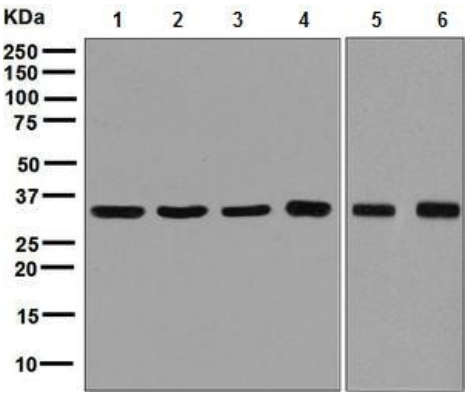
Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915) at 1/20000 dilution (Purified) + HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates at 15 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 36 kDa

Observed band size: 36 kDa



Western blot - Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915)

All lanes : Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915) at 1/10000 dilution (unpurified)

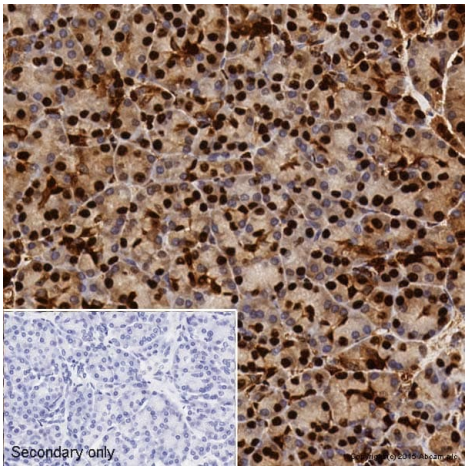
Lane 1 : HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cell lysate
Lane 2 : HeLa (human epithelial cell line from cervix adenocarcinoma) cell lysate
Lane 3 : HepG2 (human liver hepatocellular carcinoma cell line) cell lysate
Lane 4 : HUVEC (human umbilical vein endothelial cell line) cell lysate
Lane 5 : MCF7 (human breast adenocarcinoma cell line) cell lysate
Lane 6 : SH-SY5Y (human neuroblastoma cell line from bone marrow) cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP labelled Goat anti-Rabbit IgG at 1/2000 dilution

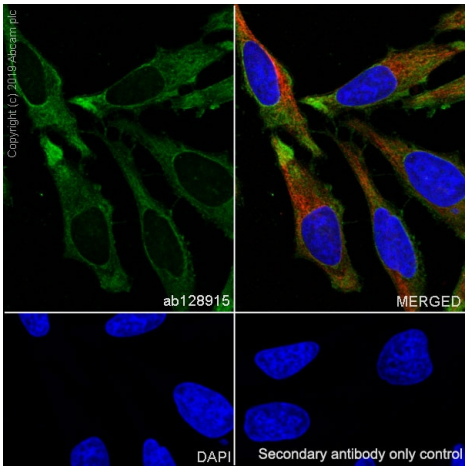
Predicted band size: 36 kDa
Observed band size: 35 kDa

Secondary antibody - **anti-rabbit HRP** (**ab6721**)



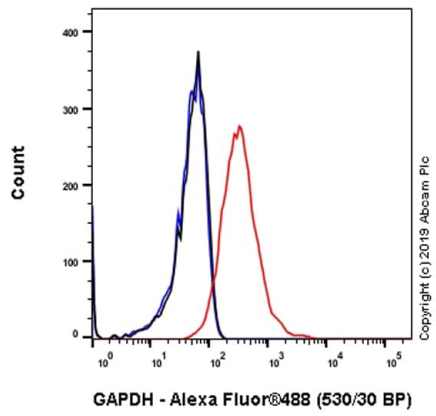
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915)

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.



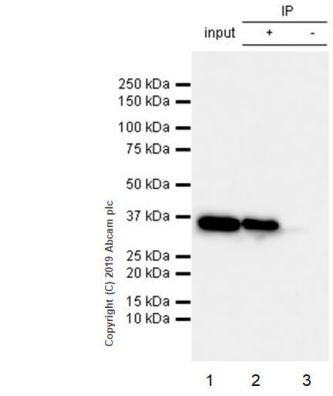
Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915)

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.



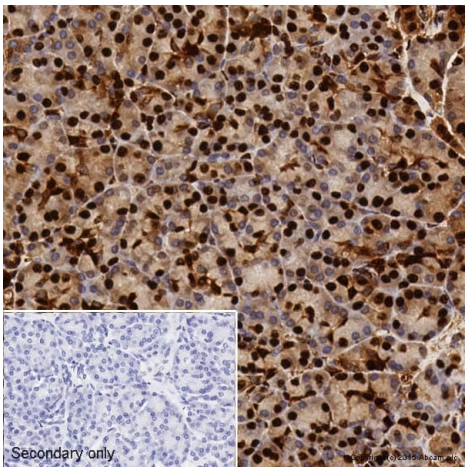
Flow Cytometry (Intracellular) - Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915)

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).



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

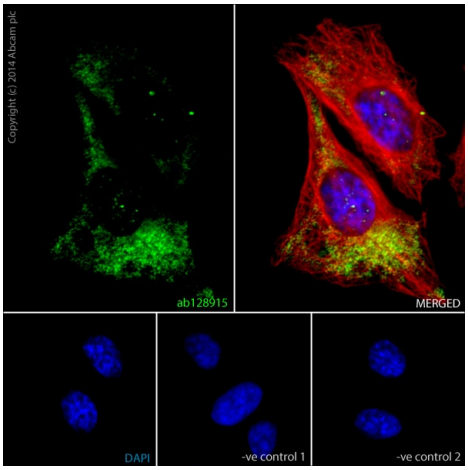
ab128915 (purified) at 1/20 dilution (0.5ug) immunoprecipitating GAPDH in HeLa whole cell lysates.
Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates 10ug
Lane 2 (+): ab128915 & HeLa whole cell lysates
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab128915 in 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915)

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,

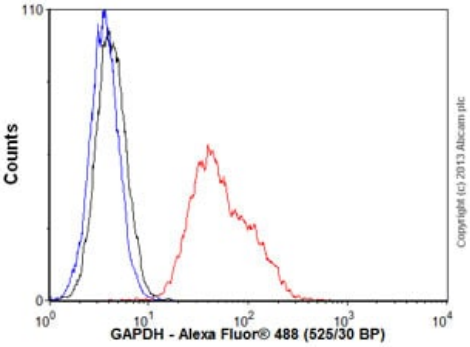
supported by the NIHR Cambridge Biomedical Research Centre



Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915)

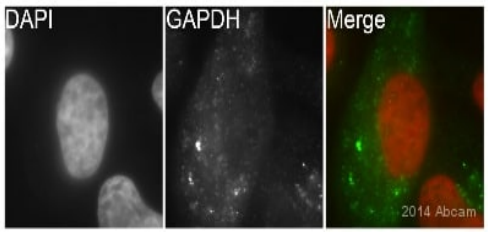
ab128915 (unpurified) staining GAPDH in HeLa (human epithelial cell line from cervix adenocarcinoma) cells. The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab128915 at 2µg/ml and **ab7291** 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.



Flow Cytometry (Intracellular) - Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915)

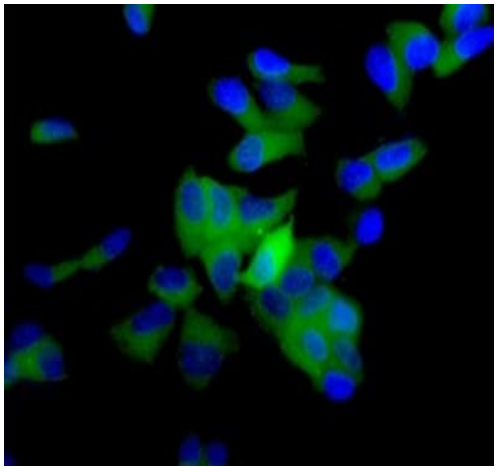
Overlay histogram showing HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained with ab128915 (unpurified) (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 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.



Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915)

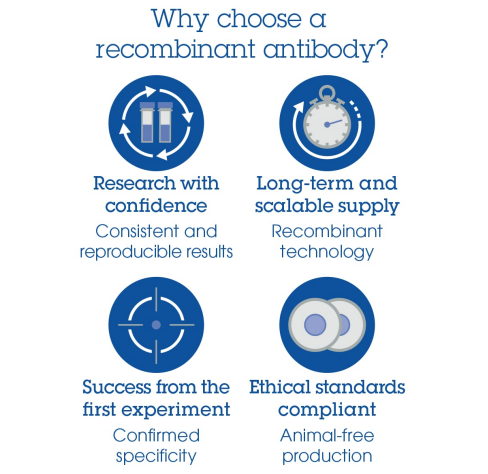
This image is courtesy of an Abreview submitted by Kirk McManus

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.



Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915)

ab128915 (unpurified), at 1/250, staining GAPDH in MCF7 (human breast adenocarcinoma cell line) cells by immunofluorescence.



Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915)

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