abcam

ab128915

Anti-GAPDH antibody [EPR6256] - Loading Control

Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915) is a rabbit monoclonal antibody detecting GAPDH in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF**. Suitable for **African green monkey, Human**.

- Biophysical QC for unrivalled batch-batch consistency
- Over 270 publications

Lab Essentials | Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR6256
Purification technique	Affinity purification Protein A
Concentration	0.122 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/10 - 1/100
Notes	-

Expected

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

WB

Tested

Species	African green monkey
Dilution info	1/10000 - 1/50000
Notes	-

Species	Human
Dilution info	1/10000 - 1/50000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/250 - 1/500
Notes	2.0 µg/ml

Expected

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

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/20
Notes	For unpurified use at 1/100 - 1/1000. ab172730 - 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	1/2000
Notes	**For unpurified use at 1/250.** Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle, Stable for 12 months at -20°C

Notes

What is this antibody validated in?
Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in African green monkey, Human samples.

What is the molecular weight of GAPDH?
Anti-GAPDH [EPR6256] - Loading Control (ab128915) is often used as a loading control in Western blot. It specifically detects a band for GAPDH (UniProt: P04406) at a molecular weight of 36kDa.

Trusted by the scientific community
Anti-GAPDH [EPR6256] - Loading Control (ab128915) was first used in a scientific publication in 2012 and has been cited over 270 times in peer-reviewed journals.

Reviewed by scientists
Anti-GAPDH [EPR6256] - Loading Control (ab128915) has over 10 independent reviews from customers.

Other related products
We have a range of other formats of antibody clone [EPR6256] also available for your convenience: ab128915, HRP - ab185059, Carrier free - ab186930, Biotin - ab195904, Alexa Fluor® 647 - ab215227, PerCP - ab216377, APC - ab221270

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

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.

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

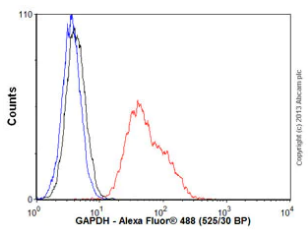
We do not recommend this combination. It is not covered by our product promise.

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.

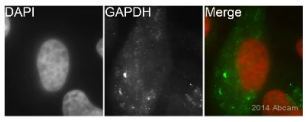
12 product images



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

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

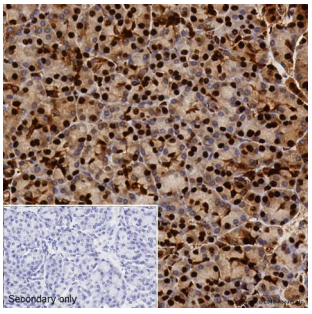
Overlay histogram showing HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained with ab128915 (unpurified) (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum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

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

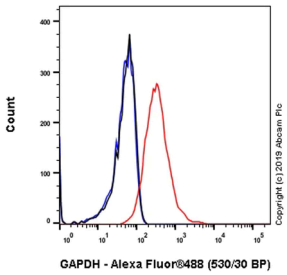
ab128915 (unpurified) staining GAPDH in human HeLa (human epithelial cell line from cervix adenocarcinoma) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde and permeabilized with 0.5% Triton X-100 in PBS. Samples were incubated with primary antibody (1/500 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.



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

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

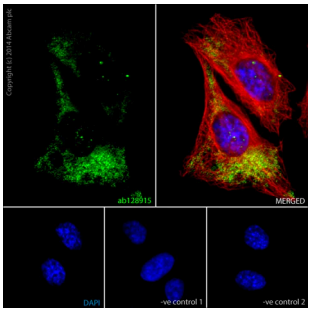
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human bladder carcinoma tissue sections labeling GAPDH with purified ab128915 at 1/2000 dilution (0.06 µg/ml). Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP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



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

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

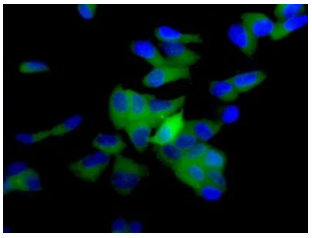
Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling GAPDH with purified ab128915 at 1/20 dilution (10µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluorr® 488, ab150077)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



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

GAPDH Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-GAPDH antibody

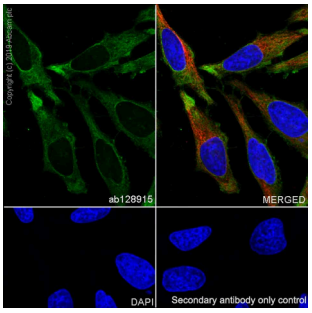
ab128915 (unpurified) staining GAPDH in HeLa (human epithelial cell line from cervix adenocarcinoma) cells. The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab128915 at 2µg/ml and ab7291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



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

GAPDH Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-GAPDH antibody

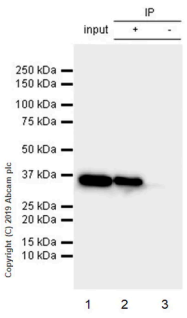
ab128915 (unpurified), at 1/250, staining GAPDH in MCF7 (human breast adenocarcinoma cell line) cells by immunofluorescence.



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

GAPDH Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-GAPDH antibody

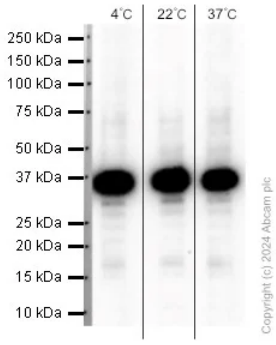
Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling GAPDH with purified ab128915 at 1/250 dilution (0.4 µg/ml). Cells were fixed in 100% Methanol. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule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



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

Predicted band size: 25 kDa, 36 kDa



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

GAPDH Western blot staining of HeLa whole cell lysate using rabbit Anti-GAPDH antibody

This blot was produced using 4-20% SDS-PAGE containing 15 µg of HeLa whole cell lysate per lane at 150V for 1hr before being transferred onto a 0.45 µm PVDF membrane at 75V for 1hr. The membrane was then blocked for 1hr using 5% NFDM/TBST, then incubated with ab128915 (1/200,000) at room temperature for 1hr. After being washed three times in TBST, the membrane was incubated with Peroxidase conjugated goat anti-rabbit IgG (H+L) (ab97051) at 1/20,000 dilution for 1hr at room temperature. The membrane was washed three times again. Then the signal was developed using the ECL technique.

ab128915 was stored at a range of temperatures (+4°C, +22°C, +37°C) for 1 week before being tested in WB. The image shows the band intensity remains relatively constant across all storage temperatures, demonstrating that antibody activity is not affected under these conditions. This data was generated as part of a structured antibody stability study supporting Abcam’ s transition from cold⊠chain to ambient shipping.

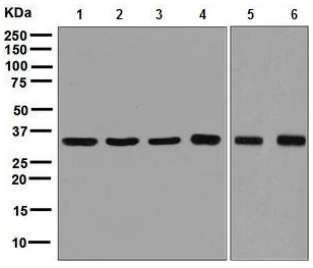
All lanes:
Western blot - Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915) at 1/200000 dilution

All lanes:
HeLa whole cell lysate at 15 µg with NDFM/TBST

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Exposure time: 60s



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

GAPDH Western blot staining using rabbit Anti-GAPDH antibody

Secondary antibody - anti-rabbit HRP (ab6721)

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

Lane 1:
HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cell lysate at 10 µg

Lane 2:
HeLa (human epithelial cell line from cervix adenocarcinoma) cell lysate at 10 µg

Lane 3:
HepG2 (human liver hepatocellular carcinoma cell line) cell lysate at 10 µg

Lane 4:
HUVEC (human umbilical vein endothelial cell line) cell lysate at 10 µg

Lane 5:
MCF7 (human breast adenocarcinoma cell line) cell lysate at 10 µg

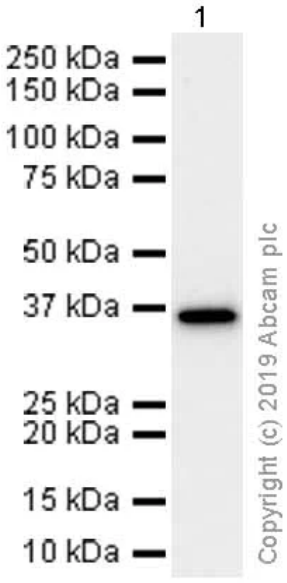
Lane 6:
SH-SY5Y (human neuroblastoma cell line from bone marrow) cell lysate at 10 µg

Secondary

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

Predicted band size: 36 kDa

Observed band size: 35 kDa



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

All lanes:
Western blot - Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915) at 1/20000 dilution

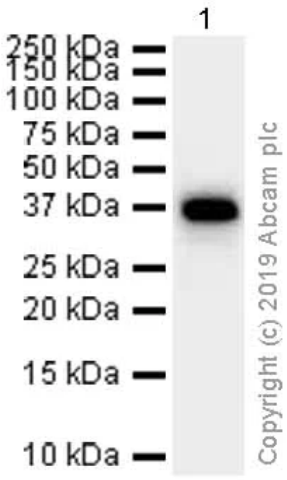
All lanes:
HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates at 15 µg

Secondary

All lanes:
Western blot - 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:
Western blot - Anti-GAPDH antibody [EPR6256] - Loading Control (ab128915) at 1/20000 dilution

All lanes:
COS-1 (African green monkey kidney fibroblast-like) whole cell lysates at 15 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 36 kDa

Observed band size: 36 kDa

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