abcam

Product datasheet

Anti-GAPDH antibody - Loading Control ab9485

★★★★★ 76 Abreviews　699 References　画像数 7

製品の概要

製品名	Anti-GAPDH antibody - Loading Control
製品の詳細	Rabbit polyclonal to GAPDH - Loading Control
由来種	Rabbit
アプリケーション	**適用あり:** IHC-P, IP, ELISA, WB, IHC-Fr, ICC/IF, Flow Cyt
種交差性	**交差種:** Mouse, Rat, Chicken, Dog, Human, Saccharomyces cerevisiae, Xenopus laevis, Schizosaccharomyces pombe, African green monkey
免疫原	Full length native protein (purified) corresponding to Human GAPDH.
ポジティブ・コントロール	WB: HeLa, A431, A549, NIH3T3, PC12 whole cell lysate ICC: U2OS cells ICC/IF: HeLa cells, NIH3T3 cells IHC/P: Hu Pancreas (FFPE)

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
バッファー	pH: 7.40 Preservative: 0.01% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
精製度	Protein A purified
ポリ/モノ	ポリクローナル
アイソタイプ	IgG

アプリケーション

Our Abpromise guarantee covers the use of **ab9485** in the following tested applications.

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

アプリケーション	Abreviews	特記事項
IHC-P		Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP	★★★☆☆	1/250.
ELISA	★★★★★	1/2500 - 1/5000.
WB	★★★★★	1/2500. Detects a band of approximately 40 kDa (predicted molecular weight: 37 kDa). Some customers have experienced that milk significantly decreases the signal in WB compared to BSA. In-house we use BSA. We recommend Goat Anti-Rabbit IgG H&L (Alexa Fluor® 790) (ab175781) secondary antibody.
IHC-Fr	★★★★★	1/250.
ICC/IF	★★★★★	Use a concentration of 5 µg/ml. We recommend Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) secondary antibody.
Flow Cyt		Use at an assay dependent concentration. ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.

ターゲット情報

機能

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.

パスウェイ

Carbohydrate degradation; glycolysis; pyruvate from D-glyceraldehyde 3-phosphate: step 1/5.

配列類似性

Belongs to the glyceraldehyde-3-phosphate dehydrogenase family.

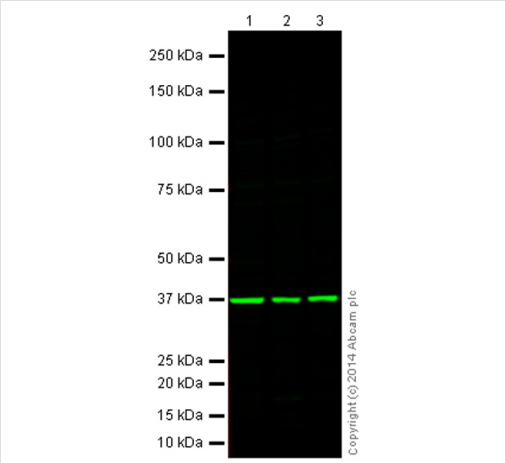
翻訳後修飾

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

細胞内局在

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.

画像



Western blot - Anti-GAPDH antibody - Loading Control (ab9485)

All lanes : Anti-GAPDH antibody - Loading Control (ab9485) at 1/2500 dilution

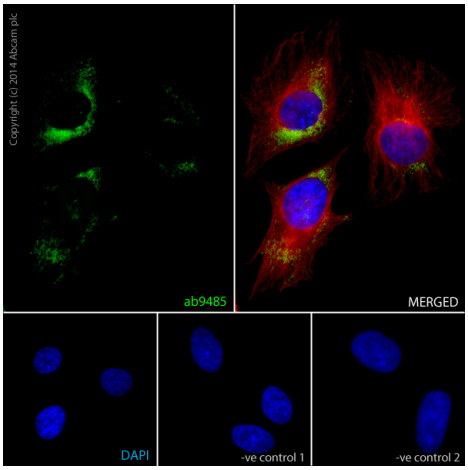
Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : A431 (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 3 : A549 (Human lung adenocarcinoma epithelial cell line) Whole Cell Lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (Alexa Fluor® 790) (ab175781) secondary antibody at 1/10000 dilution

Predicted band size: 37 kDa
Observed band size: 37 kDa

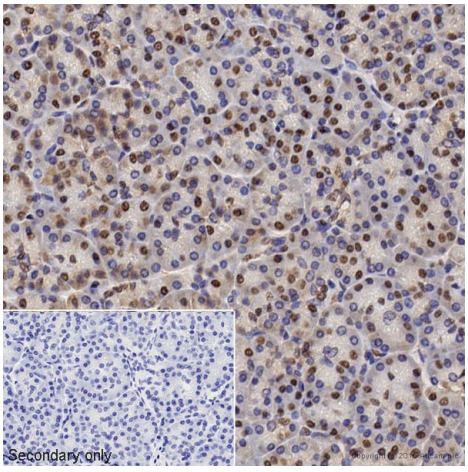
This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using Licor blocking buffer before being incubated with ab9485 overnight at 4°C. Antibody binding was detected using Goat Anti-Rabbit IgG H&L (Alexa Fluor® 790) (ab175781) secondary antibody at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.



Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody - Loading Control (ab9485)

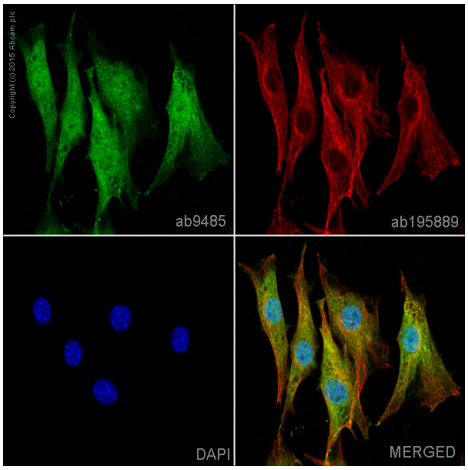
ab9485 staining GAPDH in HeLa cells. The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab9485 at 5µg/ml and ab7291 at 1µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) secondary antibody at 2 µg/ml (shown in green) and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (ab150120) secondary antibody 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.



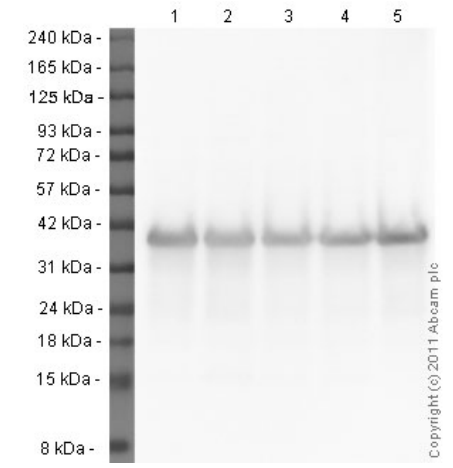
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GAPDH antibody - Loading Control (ab9485)

IHC image of ab9485 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 ab9485, 5µg/ml working concentration, 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



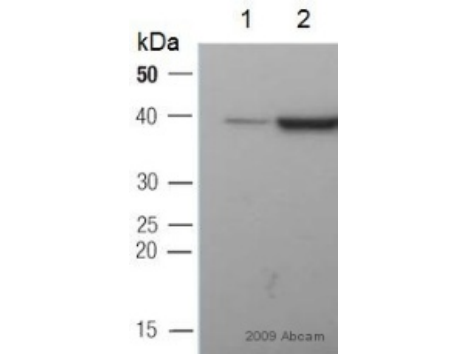
Immunocytochemistry/ Immunofluorescence - Anti-GAPDH antibody - Loading Control (ab9485)

ab9485 staining GAPDH in NIH3T3 cells. The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes 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 ab9485 at 5µg/ml and ab195889 at 1/250 overnight at +4°C, followed by a further incubation at room temperature for 1h with Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) secondary antibody at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Western blot - Anti-GAPDH antibody - Loading Control (ab9485)

Western blot image using the Optiblot Reducing Electrophoresis Kit - 10 x 10 cm (4-20%) (ab119220) with the
Prism Ultra Protein Ladder (ab116028) 5µl used. We recommend using our ECL substrate kit (ab65623) .
20ug of Lysate per lane and detection using ab9485 diluted to 1ug/ml.
Lane 1: Hela cell lysate
Lane 2: Jurkat cell lysate
Lane 3: A431 cell lysate
Lane 4: HEK293 cell lysate
Lane 5: HepG2 cell lysate.



Western blot - Anti-GAPDH antibody - Loading Control (ab9485)

This image is a courtesy of Anonymous Abreview

All lanes : Anti-GAPDH antibody - Loading Control (ab9485) at 1/2500 dilution

Lane 1 : Lysate prepared from human Huh-7 cells at 2 µg
Lane 2 : Lysate prepared from human Huh-7 cells at 20 µg

Secondary
All lanes : HRP-conjugated sheep polyclonal to rabbit IgG at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 37 kDa
Observed band size: 40 kDa
why is the actual band size different from the predicted?

Exposure time: 5 minutes

Western blot - Anti-GAPDH antibody - Loading Control (ab9485)

All lanes : Anti-GAPDH antibody - Loading Control (ab9485) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 37 kDa
Observed band size: 37 kDa

Exposure time: 10 seconds

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab9485 overnight at 4°C. Antibody binding was detected using Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody, and visualised using ECL development solution ab133406.

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