abcam

Product datasheet

HRP Anti-GAPDH antibody - Loading Control ab9385

*** 11 Abreviews 93 References 2 Images

Overview

Product name HRP Anti-GAPDH antibody - Loading Control

Description HRP Rabbit polyclonal to GAPDH - Loading Control

Host species Rabbit
Conjugation HRP

Tested applications Suitable for: WB

Species reactivity Reacts with: Human

Predicted to work with: Mouse, Rat, Cow, Saccharomyces cerevisiae, Xenopus laevis

Immunogen Full length native protein (purified) corresponding to Human GAPDH.

Positive controlThis antibody gave a positive signal in the following whole cell lysates: HeLa; Jurkat; A431;

Hek293.

General notesThe Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

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. Store In the Dark.

Storage buffer Preservative: 0.1% 10% Proclin 300 Solution

Constituents: PBS, 30% Glycerol, 1% BSA

Purity Protein A purified

Primary antibody notesThis antibody is the HRP conjugated version of <u>ab9485</u>, for more convenient use as a loading

control antibody.

Clonality Polyclonal

Isotype IgG

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Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab9385 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	★★★★ <u>(11)</u>	1/5000. Detects a band of approximately 38 kDa (predicted molecular weight: 36 kDa). Milk blocking may cause 'no bands' problem. Please try BSA as well.

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 3phosphate (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

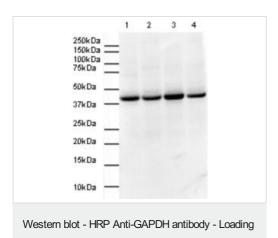
Cytoplasm > cytosol. Nucleus. Cytoplasm > perinuclear region. Membrane. Translocates to the

nucleus following S-nitrosylation and interaction with SIAH1, which contains a nuclear localization

signal (By similarity). Postnuclear and Perinuclear regions.

Images

Control (ab9385)



All lanes: HRP Anti-GAPDH antibody - Loading Control (ab9385)

at 1 µg/ml

Lane 1: HeLa cell lysate Lane 2: A431 cell lysate Lane 3: Jurkat cell lysate Lane 4: 293 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 36 kDa

Observed band size: 38 kDa

Exposure time: 1 minute

Lane 1 - 4: GAPDH antibody - Loading Control (ab9385) at 1 ug/ml

Lane 1: HeLa cell lysate at 20 ug

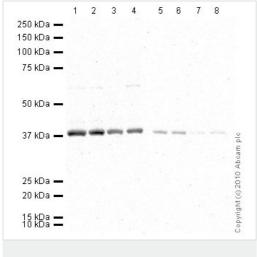
Lane 2: A431 cell lysate at 20 ug

Lane 3: Jurkat cell lysate at 20 ug

Lane 4: 293 cell lysate at 20 ug

Performed under reducing conditions.

Exposure time: 1 minute



Western blot - HRP Anti-GAPDH antibody - Loading Control (ab9385)

Lanes 1-4: HRP Anti-GAPDH antibody - Loading Control

(ab9385) at 1/5000 dilution ((Blocked in 5% BSA))

Lanes 5-8: HRP Anti-GAPDH antibody - Loading Control (ab9385) at 1/5000 dilution ((Blocked in 5% MILK))

Lanes 1 & 5: HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate (ab27252)

Lanes 2 & 6: Jurkat (Human) Whole Cell Lysate (ab52254)

Lanes 3 & 7: A-431 whole cell lysate (ab7909)

Lanes 4 & 8 : HEK-293 whole cell lysate (ab7902)

Lysates/proteins at 10 µg per lane.

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 36 kDa

The membrane 1-4 was blocked in 5% BSA (1 hour). The membrane 5-8 was blocked in 5% MILK (1 hour). Abcam routinely uses 5% BSA to block, however following recent customer feedback our labs investigated the effect of 5% milk blocking. We can now confirm that milk is not a suitable blocking agent for this antibody and significantly decreases the signal on the membrane.

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