

GAPDH Antibody

Purified Mouse Monoclonal Antibody (Mab) Catalog # AM8706b

Specification

GAPDH Antibody - Product Information

Application Primary Accession Reactivity Host Clonality Isotype Calculated MW WB, FC, IHC-P,E <u>P04406</u> Human, Mouse, Rat Mouse monoclonal IgG1,k 36053

GAPDH Antibody - Additional Information

Gene ID 2597

Other Names Glyceraldehyde-3-phosphate dehydrogenase, GAPDH, 1.2.1.12, Peptidyl-cysteine S-nitrosylase GAPDH, 2.6.99.-, GAPDH, GAPD

Target/Specificity

This GAPDH antibody is generated from a mouse immunized with a recombinant protein between 43-335 amino acids from human GAPDH.

Dilution WB~~1:8000 FC~~1:25 IHC-P~~1:25 E~~Use at an assay dependent concentration.

Format

Purified monoclonal antibody supplied in PBS with 0.09% (W/V) sodium azide. This antibody is purified through a protein G column, followed by dialysis against PBS.

Storage

Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.

Precautions

GAPDH Antibody is for research use only and not for use in diagnostic or therapeutic procedures.

GAPDH Antibody - Protein Information

Name GAPDH {ECO:0000303|PubMed:2987855, ECO:0000312|HGNC:HGNC:4141}

Function Has both glyceraldehyde-3-phosphate dehydrogenase and nitrosylase activities, thereby



playing a role in glycolysis and nuclear functions, respectively (PubMed:<u>11724794</u>, PubMed:<u>3170585</u>). 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 (PubMed:<u>11724794</u>, PubMed:<u>3170585</u>). 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:<u>23071094</u>). 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:<u>23071094</u>). 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:<u>2332158</u>, PubMed:<u>27387501</u>). Participates in nuclear events including transcription,

Cellular Location

Cytoplasm, cytosol. Nucleus {ECO:0000250|UniProtKB:P04797}. Cytoplasm, perinuclear region. Membrane Cytoplasm, cytoskeleton {ECO:0000250|UniProtKB:P04797} Note=Translocates to the nucleus following S-nitrosylation and interaction with SIAH1, which contains a nuclear localization signal (By similarity). Postnuclear and Perinuclear regions (PubMed:12829261) {ECO:0000250|UniProtKB:P04797, ECO:0000269|PubMed:12829261}

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

GAPDH Antibody - Protocols

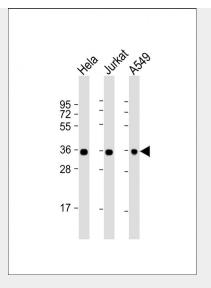
SIRT1, HDAC2 and PRKDC (By similarity).

Provided below are standard protocols that you may find useful for product applications.

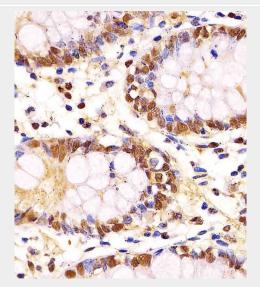
- <u>Western Blot</u>
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- <u>Cell Culture</u>

GAPDH Antibody - Images



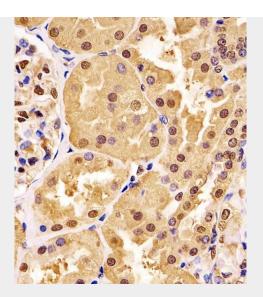


All lanes : Anti-GAPDH Antibody at 1:8000 dilution Lane 1: Hela whole cell lysate Lane 2: Jurkat whole cell lysate Lane 3: A549 whole cell lysate Lysates/proteins at 20 μ g per lane. Secondary Goat Anti-mouse IgG, (H+L), Peroxidase conjugated at 1/10000 dilution. Predicted band size : 36 kDa Blocking/Dilution buffer: 5% NFDM/TBST.

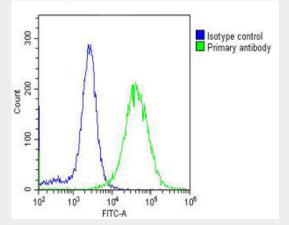


AM8706b staining GAPDH in human colon tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 3% BSA for 0. 5 hour at room temperature; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody (1/25) for 1 hours at 37°C. A undiluted biotinylated goat polyvalent antibody was used as the secondary antibody.





AM8706b staining GAPDH in human kidney tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 3% BSA for 0. 5 hour at room temperature; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody (1/25) for 1 hours at 37°C. A undiluted biotinylated goat polyvalent antibody was used as the secondary antibody.



Overlay histogram showing Hela cells stained with AM8706b(green line). The cells were fixed with 2% paraformaldehyde (10 min) and then permeabilized with 90% methanol for 10 min. The cells were then icubated in 2% bovine serum albumin to block non-specific protein-protein interactions followed by the antibody (AM8706b, 1:25 dilution) for 60 min at 37°C. The secondary antibody used was Goat-Anti-Mouse IgG, DyLight® 488 Conjugated Highly Cross-Adsorbed(OJ192088) at 1/200 dilution for 40 min at 37°C. Isotype control antibody (blue line) was mouse IgG1 (1 μ g/1x10^6 cells) used under the same conditions. Acquisition of >10, 000 events was performed.

GAPDH Antibody - Background

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. Modulates the organization and assembly of the cytoskeleton. Facilitates the CHP1-dependent microtubule and membrane associations through its ability to stimulate the binding of CHP1 to microtubules (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. Component of the GAIT (gamma interferon- activated inhibitor of translation) complex which mediates interferon-gamma-induced transcript-selective translation inhibition in inflammation processes. 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.

GAPDH Antibody - References

Hanauer A., et al.EMBO J. 3:2627-2633(1984). Arcari P., et al.Nucleic Acids Res. 12:9179-9189(1984). Tso J.Y., et al.Nucleic Acids Res. 13:2485-2502(1985). Tokunaga K., et al.Cancer Res. 47:5616-5619(1987). Allen R.W., et al.J. Biol. Chem. 262:649-653(1987).