

Product Datasheet

GAPDH Antibody NB300-324

Unit Size: 0.1 ml

Store at 4C. Do not freeze.

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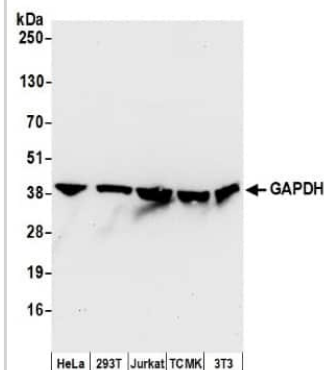
NB300-324

GAPDH Antibody

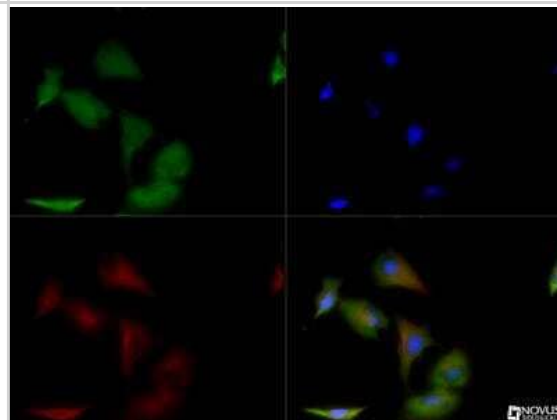
Product Information	
Unit Size	0.1 ml
Concentration	0.2 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.09% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	TBS and 0.1% BSA
Target Molecular Weight	36 kDa
Product Description	
Host	Rabbit
Gene ID	2597
Gene Symbol	GAPDH
Species	Human, Mouse, Xenopus
Reactivity Notes	Human and mouse. Based upon 100% sequence identity, this antibody is predicted to react with Rat, Chicken, Turkey, Guinea pig, Pig, Rhesus Monkey, Gorilla, Chimpanzee, West Indian ocean coelacanth, Neobatrachia, Sauropsida, Common iguana, Black-banded sea krait, Domestic pigeon and Indian bull frog.
Marker	Cytosolic Marker
Immunogen	This GAPDH antibody was developed against an epitope between residues 250 and 300 of human GAPDH [accession number NP_002037.2]
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Western Blot 1:2000-1:10000, Simple Western 1:25, Immunohistochemistry, Immunocytochemistry/ Immunofluorescence 1:50, Immunoprecipitation, Immunohistochemistry-Paraffin 1:100 - 1:500
Application Notes	<p>This GAPDH antibody is useful for Immunohistochemistry-paraffin, Immunocytochemistry/Immunofluorescence and Western blot applications. In WB, a specific band is observed at approx. 36 kDa molecular weight position. In Immunohistochemistry, epitope retrieval with citrate buffer pH6.0 is recommended for formalin-fixed paraffin-embedded tissue sections.</p> <p>In Simple Western only 10 - 15 uL of the recommended dilution is used per data point.</p> <p>See Simple Western Antibody Database for Simple Western validation: Tested in HeLa lysate 1.0 mg/mL, separated by Size, antibody dilution of 1:25, apparent MW was 43 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.</p> <p>The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.</p>

Images

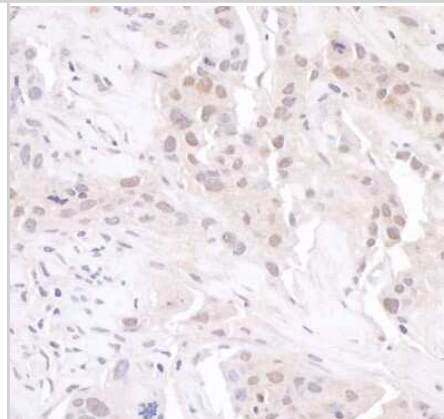
Western Blot: GAPDH Antibody [NB300-324] - Detection of Human and Mouse GAPDH by Western Blot. Theoretical molecular weight: 36 kDa. Samples: Whole cell lysate (50 ug) from HeLa, 293T, Jurkat, mouse TCMK-1, and mouse NIH3T3 cells prepared using NETN lysis buffer. Antibody: Affinity purified rabbit anti-GAPDH antibody NB300-324 used for WB at 0.04 ug/ml. Detection: Chemiluminescence with an exposure time of 10 seconds.



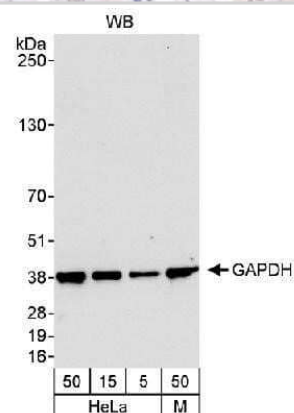
Immunocytochemistry/Immunofluorescence: GAPDH Antibody [NB300-324] - GAPDH antibody was tested in Hela cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



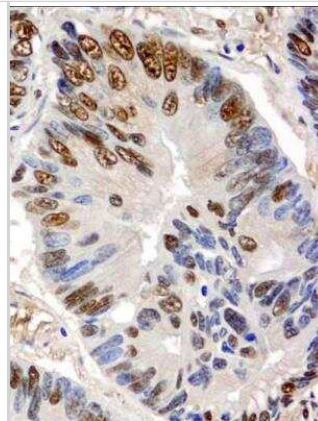
Immunohistochemistry: GAPDH Antibody [NB300-324] - Detection of human GAPDH by immunohistochemistry. Sample: FFPE section of human breast carcinoma. Antibody: Affinity purified rabbit anti-GAPDH (NB100-79808). Detection: DAB



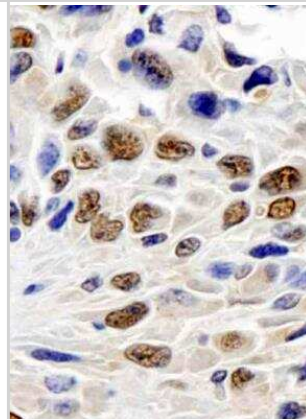
Western Blot: GAPDH Antibody [NB300-324] - Detection of Human and Mouse GAPDH, theoretical molecular weight 36 kDa. Whole cell lysate from HeLa (5, 15 and 50 mcg) and mouse NIH3T3 (M; 50 mcg). Affinity purified rabbit anti-GAPDH antibody used at 0.04 mcg/ml. Chemiluminescence with an exposure time of 10 seconds.



Immunohistochemistry-Paraffin: GAPDH Antibody [NB300-324] - IHC detection of GAPDH in formalin-fixed paraffin-embedded human lung carcinoma tissue sections with NB300-324 at a dilution of 1:200



Immunohistochemistry-Paraffin: GAPDH Antibody [NB300-324] - IHC detection of GAPDH in formalin-fixed paraffin-embedded mouse squamous cell carcinoma tissue sections with NB300-324 at a dilution of 1:200.



Simple Western: GAPDH Antibody [NB300-324] - Simple Western lane view shows a specific band for GAPDH in 1.0 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system. Note: band observed higher than predicted molecular weight of 36 kDa.



Publications

Christensen CL, Kan SH, Andrade-Heckman P, Rha AK et Al. Base editing rescues acid β -glucosidase function in infantile-onset Pompe disease patient-derived cells *Mol Ther Nucleic Acids* 2024-07-01 [PMID: 38948331]

Rha AK, Kan SH, Andrade-Heckman P et Al. Base editing of the GLB1 gene is therapeutic in GM1 gangliosidosis patient-derived cells *Mol Genet Metab* 2024-10-14 [PMID: 39303319]

Chiang YP, Li Z, He M et al. Sphingomyelin synthase-related protein SMSr is a phosphatidylethanolamine phospholipase C that promotes nonalcoholic fatty liver disease *Journal of Biological Chemistry* 2023-08-14 [PMID: 37586586] (Western Blot, Mouse)

Harb JF, Christensen CL, Kan SH et al. Base editing corrects the common Salla disease SLC17A5 c.115C>T variant *Molecular therapy. Nucleic acids* 2023-12-12 [PMID: 37727271] (WB)

Li Z, He M, Chen G et al. Effect of Total Sphingomyelin Synthase Activity on Low Density Lipoprotein Catabolism in Mice *bioRxiv : the preprint server for biology* 2023-02-07 [PMID: 36798262] (WB, Mouse)

Li Z, Chiang Y, He M Et al. Liver sphingomyelin synthase 1 deficiency causes steatosis, steatohepatitis, fibrosis, and tumorigenesis: An effect of glucosylceramide accumulation *iScience* 2021-12-01 [PMID: 34927020] (WB, Mouse)

Moncini S, Lunghi M, Valmadre A et al. The miR-15/107 Family of microRNA Genes Regulates CDK5R1/p35 with Implications for Alzheimer's Disease Pathogenesis *Mol. Neurobiol.* 2016-06-24 [PMID: 27343180] (WB, Human)

Jiang H, Hurt KJ, Breen K et al. Sex-specific dysregulation of cysteine oxidation and the methionine and folate cycles in female cystathionine gamma-lyase null mice: a serendipitous model of the methylfolate trap. *Biol Open* 2015-08-14 [PMID: 26276101] (WB, Mouse)



Procedures

Western Blot protocol for GAPDH Antibody (NB300-324)

GAPDH Antibody:

Western Blot Protocol

1. Perform SDS-PAGE (4-12% MOPS) on samples to be analyzed, loading 25 ug of total protein per lane.
 2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
 3. Rinse membrane with dH₂O and then stain the blot using Ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
 4. Rinse the blot in TBS for approximately 5 minutes.
 5. Block the membrane using 5% NFDM + 1% BSA in TBS + Tween, 1 hour at RT.
 6. Rinse the membrane in dH₂O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
 7. Dilute the rabbit anti-GAPDH primary antibody (NB300-324) in blocking buffer and incubate 1 hour at room temperature.
 8. Rinse the membrane in dH₂O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
 9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
 10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each (this step can be repeated as required to reduce background).
 11. Apply the detection reagent of choice in accordance with the manufacturers instructions (Pierce ECL).
- Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

Immunocytochemistry/Immunofluorescence protocol for GAPDH Antibody (NB300-324)

GAPDH Antibody:

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.



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Products Related to NB300-324

NBL1-10967	GAPDH Overexpression Lysate
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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