Product Datasheet

GAPDH Antibody
NB300-325

Unit Size: 0.1 ml

Store at 4C. Do not freeze.

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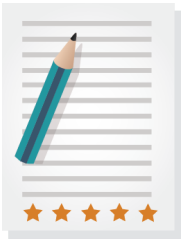
Updated 12/20/2023 v.20.1



NB300-325

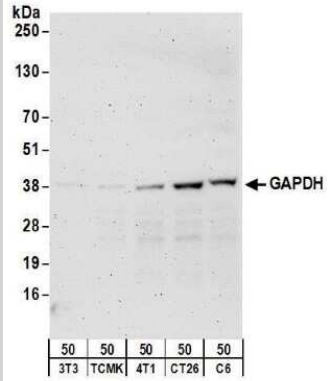
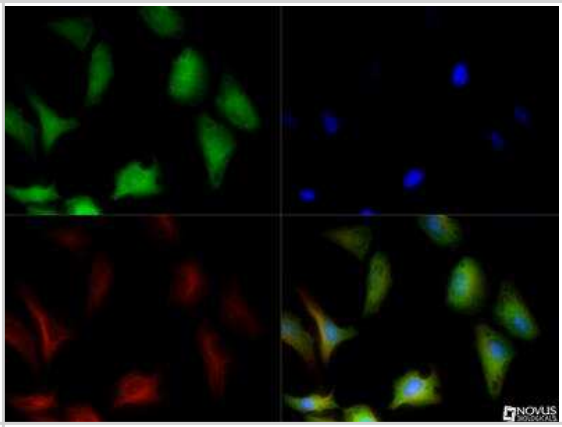
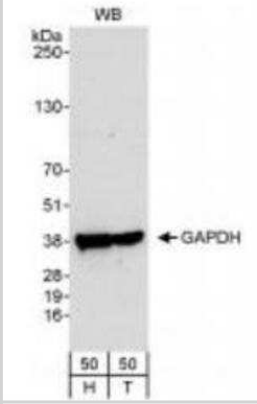
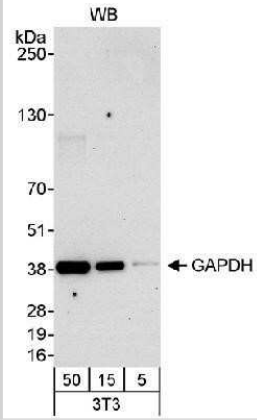
GAPDH Antibody

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

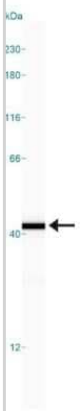
Product Description	
Host	Rabbit
Gene ID	2597
Gene Symbol	GAPDH
Species	Human, Mouse, Rat
Reactivity Notes	Human and mouse.
Marker	Cytosolic Marker
Immunogen	This GAPDH antibody was developed against an epitope between residue 300 and the C-terminus of the human GAPDH protein [accession number NP_002037.2]

Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation
Recommended Dilutions	Western Blot 1:2000-1:10000, Simple Western 1:500, Immunocytochemistry/ Immunofluorescence 1:250, Immunoprecipitation
Application Notes	This GAPDH antibody is useful for Immunocytochemistry/Immunofluorescence and Western blot, where a band is observed approx. 36 kDa. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue. 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.

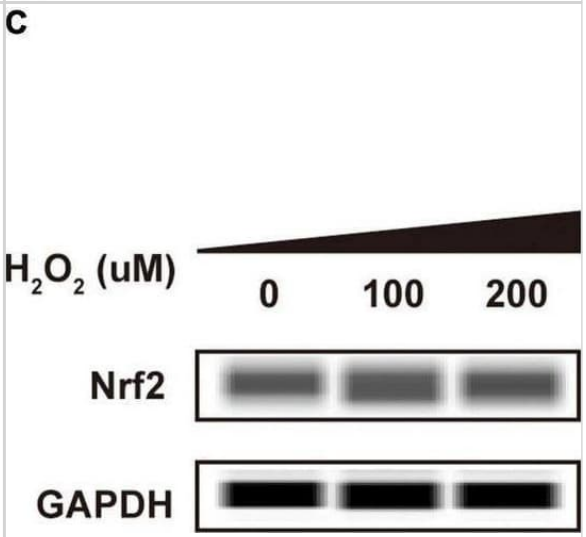
Images

Western Blot: GAPDH Antibody [NB300-325] - Detection of mouse and rat GAPDH by western blot. Samples: Whole cell lysate (50 ug) from NIH 3T3, TCMK-1, 4T1, CT26.WT, and rat C6 cells. Antibodies: Affinity purified rabbit anti-GAPDH antibody NB300-325 used for WB at 0.5 ug/ml. Detection: Chemiluminescence with an exposure time of 3 minutes.	
Immunocytochemistry/Immunofluorescence: GAPDH Antibody [NB300-325] - GAPDH antibody was tested in Hela cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).	
Western Blot: GAPDH Antibody [NB300-325] - Whole cell lysate (50 ug) from HeLa (H) and 293T (50 ug) probed with GAPDH Antibody diluted at 0.04 ug/ml	
Western Blot: GAPDH Antibody [NB300-325] - Detection of Mouse GAPDH Whole cell lysate (5, 15 and 50 ug) from mouse NIH3T3 cells. Another Affinity purified rabbit anti-GAPDH used at 0.04 mcg/ml. Chemiluminescence with an exposure time of 30 seconds.	

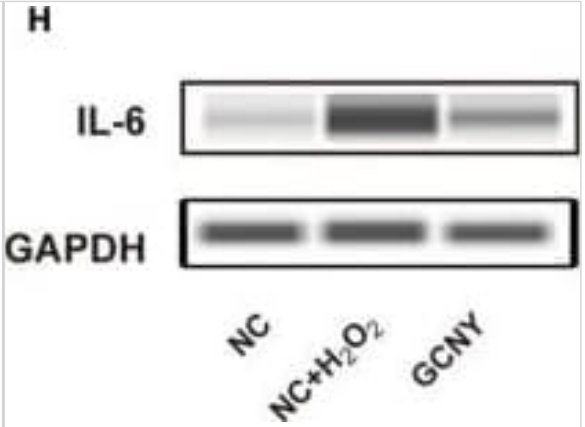
Simple Western: GAPDH Antibody [NB300-325] - Simple Western lane view shows a specific band for GAPDH in 0.05 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



WNT11 is induced by hypoxia or hypoxic mimetics in different cell types. (A) Increased Wnt11 mRNA in EMSC adipocytes (Day 12) after hypoxia-mimetic treatments. EMSC adipocytes were treated with CoCl2 (0.1 mM), DFO (0.1 mM) or DMOG (0.1 mM) for 24 hrs. Values were normalized to Tbp mRNA and are expressed relative to control (n = 3). (B,C) Increased Wnt11 mRNA by hypoxia in EMSC preadipocytes and adipocytes (Day 0–12 after differentiation) (B), and C2C12 myoblast and myocyte (Day 0 and 8 after differentiation) (C). Wnt11 mRNA was assessed by quantitative PCR in cells exposed to air (21% O2) or hypoxia (1% O2) for 24 hrs. (n = 4). Values were normalized to Tbp mRNA and are expressed relative to 21% O2 samples (left panel). (D) Immunoblot analyses of HeLa cells under normal air or hypoxia for 24 hrs. (E,F) Induction of Wnt11 by increasing concentrations of DMOG in MDA-MB-231 cells (E) and 4T1 cells (F). (G) EMSCs treated with 0.1 mM DMOG for the indicated times. Wnt11 and Vegf mRNA expression was measured by qPCR and normalized to Tbp mRNA (n = 4). (H) WNT11 protein levels after DMOG treatment normalized to α-Tubulin (upper panel; n = 4). Representative immunoblots of EMSCs treated with 0.1 mM DMOG for the indicated times (Lower panel). (I) Protein expression in MDA-MB-231 cells treated with 0.1 mM DMOG. (J) Induction of Wnt11 promoter activity by hypoxia or hypoxia mimetics. pGL3-Wnt11 promoter plasmid was transfected into C2C12 cells. Cells were incubated with DMOG (left panel, n = 4) or under 21% O2 or 1% O2 (right panel, n = 8) for 24 hrs. For panels (A–C,G,H,J), values are mean ± s.e.m. *p < 0.05, **p < 0.01. For panels of immunoblotting, laminin, α-tubulin, and ERK were used as loading controls, WNT11 normalized to α-Tubulin was shown. Image collected and cropped by CiteAb from the following open publication (https://www.nature.com/articles/srep21520), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Primary human grade III astrocytoma xenotransplant.(A) T2-weighted MRI (coronal) visualizes advanced tumor in the right hemisphere of the brain. (B) Correlative dynamic PET image (coronal) of same advanced tumor in the right hemisphere of the brain, with [18F]PBR06 uptake primarily confined to the tumor and co-localizing with tumor tissue visualized by MRI. Top arrows indicate tumor and infiltration into left hemisphere. (C) Fused MRI (A) PET (B) image. (D) Time-activity curves of injected [18F]PBR06 in tumor (green) and contralateral brain (blue). Correlative vimentin immunohistochemistry: (E) Gross; (F) Tumor + White Matter Tract (40X); (G) Tumor (40X). (H) Correlative dynamic PET image, axial view along yellow line in (B); arrows indicate tumor and infiltration into left hemisphere. Correlative TSPO immunohistochemistry: (I) Gross; (J) Tumor + White Matter Tract (40X); (K) Tumor (40X). (L) Dynamic PET image (axial) of control cohort. Correlative CD68 immunohistochemistry: (M) Gross; (N) Tumor + White Matter Tract (40X); (O) Tumor (40X). Image collected and cropped by CiteAb from the following open publication (https://dx.plos.org/10.1371/journal.pone.0141659), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Chen Y, Wang B, Lai WF et al. Chinese herbal formula (GCNY)-medicated serum alleviates peroxidation induced by H(2)O(2) in human microglial cells Frontiers in Neuroscience 2022-09-14 [PMID: 36188472] (B/N, WB)

Brinks J, van Dijk EHC, Kielbasa SM et al. The Cortisol Response of Male and Female Choroidal Endothelial Cells: Implications for Central Serous Chorioretinopathy The Journal of clinical endocrinology and metabolism 2021-09-21 [PMID: 34546342] (WB, Human)

Ahmed AA, Neidle S A G-Quadruplex-Binding Small Molecule and the HDAC Inhibitor SAHA (Vorinostat) Act Synergistically in Gemcitabine-Sensitive and Resistant Pancreatic Cancer Cells Sci Adv 2020-11-01 [PMID: 33227941] (WB, Human)

Rosen MB, Jeffay SC, Nichols HP et al. ATP Binding Cassette Sub-family Member 2 (ABCG2) and Xenobiotic Exposure During Early Mouse Embryonic Stem Cell Differentiation. Birth Defects Res. 2017-10-09 [PMID: 28990372] (WB, Mouse)

Procedures

Western Blot protocol for GAPDH Antibody (NB300-325)
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 (NB300-325) 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 (NB300-325)
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 1:25,0000 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 NB300-325

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

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