Product Datasheet

HIF-1 alpha Antibody (H1alpha67)
NB100-105

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

Reviews: 45   Publications: 1038

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Updated 7/23/2024 v.20.1

NB100-105
HIF-1 alpha Antibody (H1alpha67)

Product Information

Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	H1alpha67
Preservative	0.05% Sodium Azide
Isotype	IgG2b
Purity	Protein G purified
Buffer	PBS, 1% BSA
Target Molecular Weight	93 kDa

Product Description

Host	Mouse
Gene ID	3091
Gene Symbol	HIF1A
Species	Human, Mouse, Rat, Porcine, Bovine, Canine, Feline, Ferret, Monkey, Primate, Rabbit, Sheep, Xenopus
Reactivity Notes	Use in Rat reported in scientific literature (PMID:33816617).
Immunogen	This HIF-1 alpha Antibody (H1alpha67) was developed against a fusion protein containing amino acids 432 - 528 of human HIF-1 alpha [Uniprot# Q16665].

Product Application Details

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Applications	Western Blot, Simple Western, Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Gel Super Shift Assays, Immunoassay, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, In vitro assay, Immunoprecipitation, Ligand Activation, Proximity Ligation Assay, Tissue Culture Substratum, Chromatin Immunoprecipitation (ChIP), Immunohistochemistry Free-Floating, Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 1:500, Simple Western, Chromatin Immunoprecipitation 1 - 5 ug/IP. Use reported in scientific literature, Flow Cytometry 1:10 - 1:1000, ELISA 1:100 - 1:2000. Use reported in scientific literature (PMID 20042684), Immunohistochemistry 1:20-1:50, Immunocytochemistry/ Immunofluorescence 1:50, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:20-1:50, Immunohistochemistry-Frozen 1:20-1:50, Immunoassay reported in scientific literature (PMID 26147748), Immunoblotting reported in multiple pieces of scientific literature, In vitro assay reported in multiple pieces of scientific literature, Gel Super Shift Assays 1:1 - 1:100. Use reported in scientific literature (PMID 22411794), Proximity Ligation Assay reported in scientific literature (PMID 27595394), Tissue Culture Substratum, Ligand Activation reported in scientific literature (PMID 26147748), Immunohistochemistry Free-Floating reported in scientific literature (PMID 33242463), Chromatin Immunoprecipitation (ChIP) 1-5 ug/IP, Knockout Validated reported in scientific literature (PMID 26861754), Knockdown Validated reported in scientific literature (PMID 32772041)

| Application Notes	In WB, a band can be seen at 120 kDa representing HIF-1 alpha in induced tissues and cells. Multiple bands may be seen at 100-120 kDa representing post-translational modification of HIF-1 alpha. For WB, testing on nuclear extracts is recommended. We recommend the use of a highly sensitive ECL reagent, such as West Pico PLUS, for Western blot detection. See Simple Western Antibody Database for Simple Western validation: separated by size

Images

HIF-1 alpha induction by CoCl2 on Caki-1 cell lysate. WB image submitted by a verified customer review.
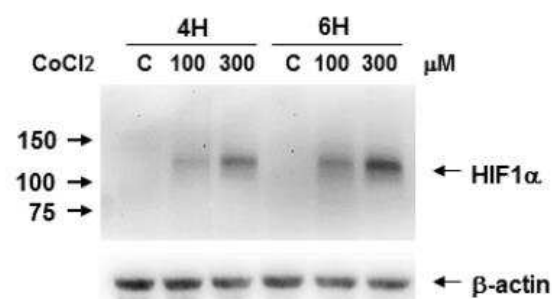

HIF-1 alpha was detected in immersion fixed DFO treated Hela cells (left) but was not detected in HIF-1 knockout HeLa cells (right) using Mouse Anti-human HIF-1 alpha monoclonal antibody (Catalog # NB100-105) at 25 ug/mL for 3 hours at room temperature. Cells were stained using a NorthernLights (TM) 557-conjugated Donkey Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to nuclei.
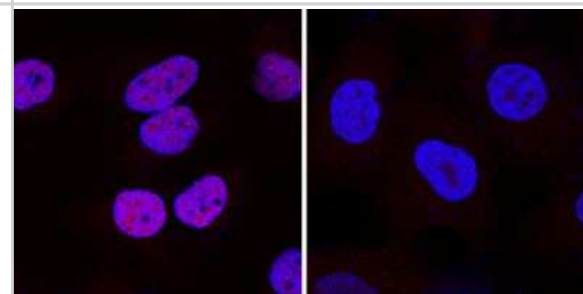

Naive CD4 T cells from WT, VHL-deficient (Vhl KO), or HIF-1 alpha-deficient (HIF-1 alpha KO) mice were differentiated under IL-22-skewing conditions for a total of 60 h. Some cells remained at normoxia for the duration of the culture (N); others were at normoxia for 35 h and then hypoxia (1% O2) for 24 h (H). At 60 h, nuclear extracts were harvested, and HIF-1 alpha and Lamin B1 levels were analyzed by Western blot. Image from verified customer review.
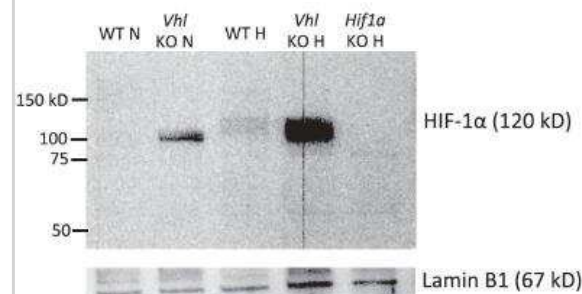
Analysis using the Alexa Fluor (R) 488 conjugate of NB100-105. Staining of HIF-1 alpha in multiple myeloma cells: H929 cells (0.5 x 10^6) were stained with Alexa Fluor 488(R) conjugated HIF-1 alpha antibody (NB100-105AF488). Image courtesy of Dr. Barbara Muz at Washington University in St. Louis School of Medicine.
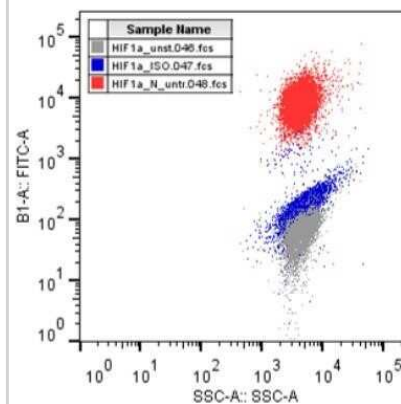

Staining of HIF-1 alpha in human kidney using NB100-105. Renal tubular epithelium showed moderate membranous, cytoplasmic and nuclear staining, and glomeruli showed faint to moderate nuclear staining.
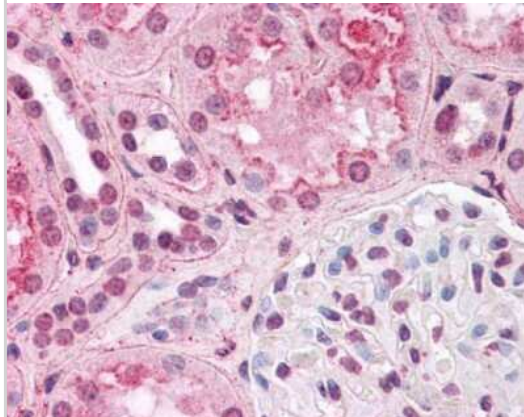

HIF1alpha/PFKFB3 is upregulated in beta-cells of HIP rats and humans with type 2 diabetes. Representative Western blot of PFKFB3 and HIF1alpha levels in nuclear-enriched- and whole cell extracts from non-diabetic (ND) and T2D donor islets. Data are presented as mean +/- SEM, n = 3 independent biological samples for each group. Statistical significance was analyzed by Student t-test (*p < 0.05, ***p < 0.001) Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/s41467-019-10444-1) licensed under a CC-BY license.
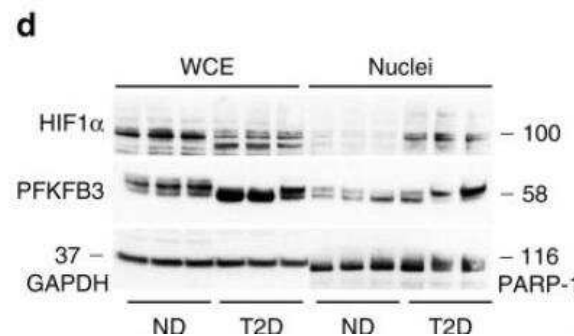

Analysis using the biotin conjugate of NB100-105. Staining of human glioblastoma multiforme.
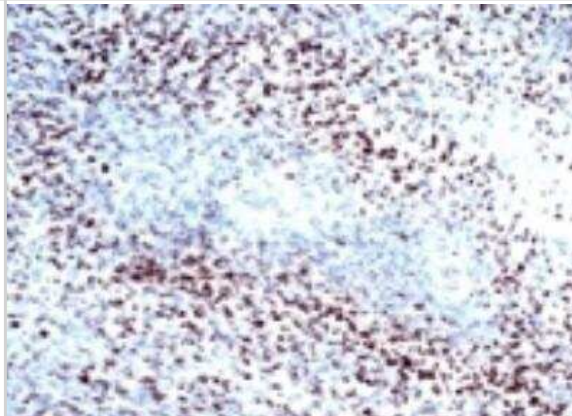
HIF-1 alpha was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line treated with DFOA using 1 ug/mL of mouse anti-HIF-1 alpha monoclonal antibody NB100-105. Cells were stained using a donkey anti-rabbit secondary antibody and counterstained with DAPI (blue).
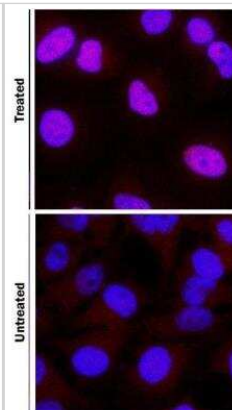

Upregulation of HIF-1alpha in human PCa. Protein expression of HIF-1alpha, VEGF, and GLUT4 were examined with western blot, in PC-3, DU145, and LNCaP cells after various treatments as indicated. Data are expressed as mean +/- SD of seven independent experiments. $p < 0.05 versus RWPE-1 or BPH1 cells or normal tissue. *p < 0.05 versus control group. #p < 0.05 versus si-HIF-1alpha or DDP group. Original blots are shown in Supplementary Figure 5. C: Ctrl; D: DDP; S: si-HIF-1alpha; D/S: DDP/si-HIF-1alpha. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/s41598-017-07973-4), licensed under a CC-BY license.
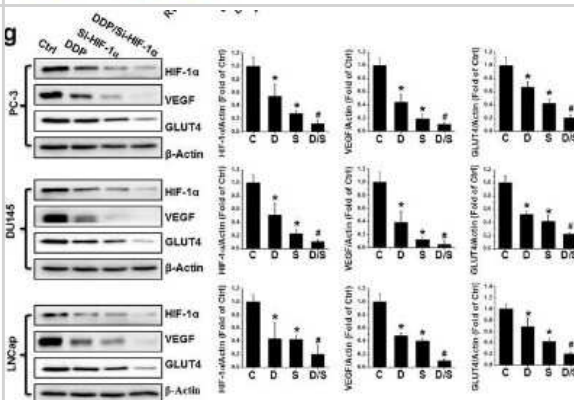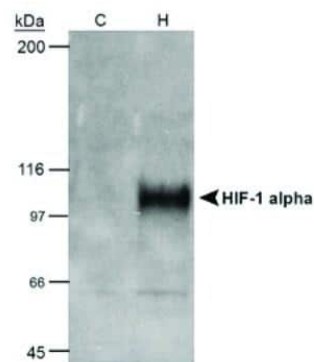

Analysis using HIF-1 alpha Antibody on human throat cancer tissue. PFA fixed paraffin-embedded sections. Primary antibody dilution 1:100. Image from verified customer review.
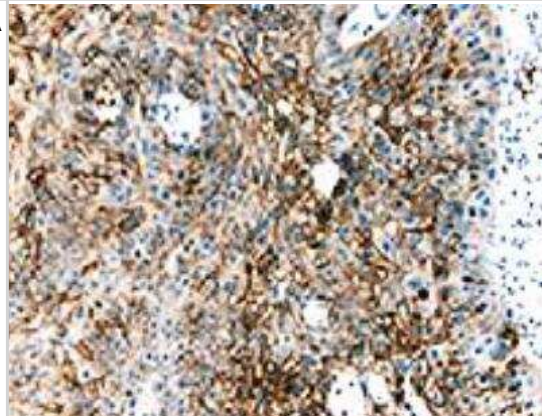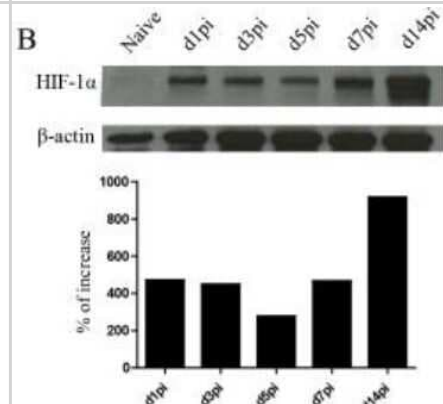

Analysis of HIF-1 alpha in human hepatocytes from cancer patient using anti-HIF-1 alpha antibody NB100-105. Western blot image submitted by a verified customer review.
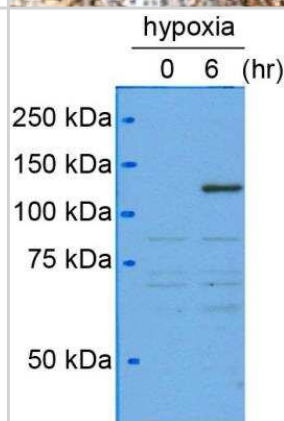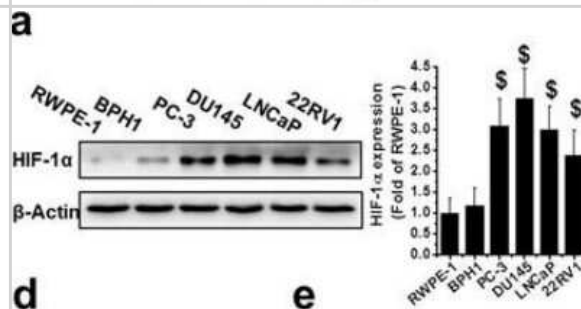
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Analysis using the HRP conjugate of NB100-105. Detection of 50 ug cobalt chloride induced COS-7 nuclear extracts (NB800-PC26) using NB100-105.	
L. donovani infection induces HIF-1 alpha expression in CD11chi splenic DCs in an IRF-5 dependent manner. Mice were infected with 2 x 10^7 amastigotes intravenously. Immunoblot analysis of HIF-1 alpha expression in CD11c+ cells from C57BL/6 mice (upper panel) and densitometric analysis normalized to beta-actin expression and expressed as fold increase to results obtained with naive mice (lower panel). Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.ppat.1004938), licensed under a CC-BY license.	
Upregulation of HIF-1alpha in human Pca. HIF-1alpha protein was detected by western blot in nonmalignant (RWPE-1 and BPH1) and PCa cell lines (PC-3, DU145, LNCaP, and 22RV1) as indicated. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/s41598-017-07973-4), licensed under a CC-BY license.	
HIF-1alpha expression in PDAC and adjacent normal pancreatic tissue. PDAC with weak (intensity 1) nuclear and cytoplasmic HIF-1alpha staining (left). PDAC with strong (intensity 3) nuclear and moderate (intensity 2) cytoplasmic HIF-1alpha staining (right). Image collected and cropped by CiteAb from the following publication (https://wjso.biomedcentral.com/articles/10.1186/s12957-018-1432-4), licensed under a CC-BY license.	
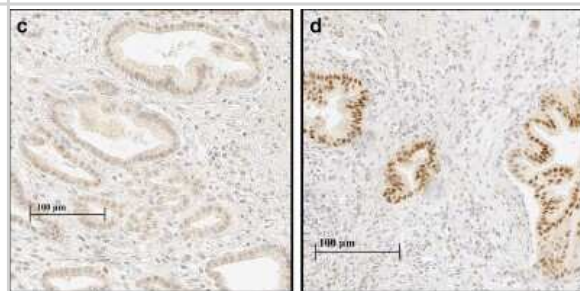
Analysis using the Alexa Fluor(R) 488 conjugate of NB100-105. Staining of HIF-1 alpha in H929 cells using HIF-1 alpha antibody. Flow cytometry image submitted by a verified customer review.
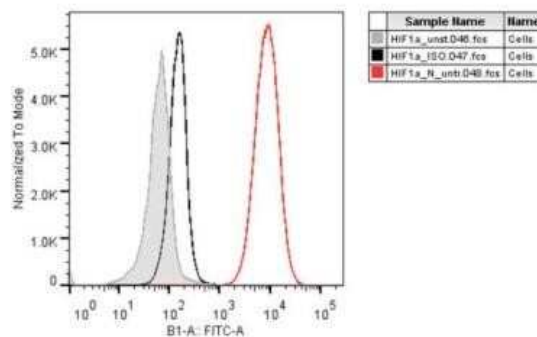
Effects of one- and five-dosage dry needling (1D, 5D) on iNOS, HIF-1α, COX-2, and VEGF expressions in a needling-treated muscle. (a) Representative Western blot images. T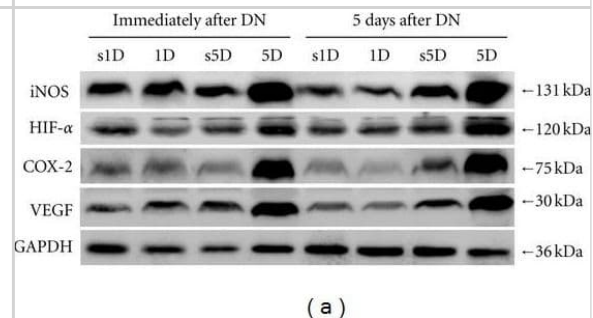he quantification of the protein levels for (b) iNOS, (c) HIF-1α, (d) COX-2, and (e) VEGF. Values are expressed as mean ± SD. *Indicates the significant difference (P < 0.05) between the sham groups (s1D and s5D). #Represents the significant difference (P < 0.05) between the 1D and 5D groups. DN: dry needling.

Hypoxia is implicated in the adaptive response after short-term bevacizumab treatment. Expression of HIF-1α in pulmonary tumor nodules of the four groups. (A) A representative western blot is shown. β-actin was used as a loading control. (B) While most tumors showed little expression of HIF-1α protein in PBS and cisplatin groups, mice that received bevacizumab and bevacizumab + cisplatin therapy showed a markedly increased level of HIF-1α expression.. *P < 0.05, **P < 0.01.
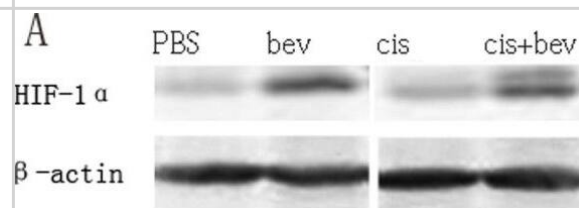
Effects of F2 on tumor-related biomarkers in vivoImmunohistochemical analysis of tumor-related proteins (HIF-1α, VEGF, MMP-2, CD31 and KI67) in tumor sections from U87 xenograft mice treated with F2 (mg/kg i.g.). Red arrows indicate the related protein detected in the tissue samples. Values are means ± SEM of three experiments. * P<0.05 compared with DDW group.
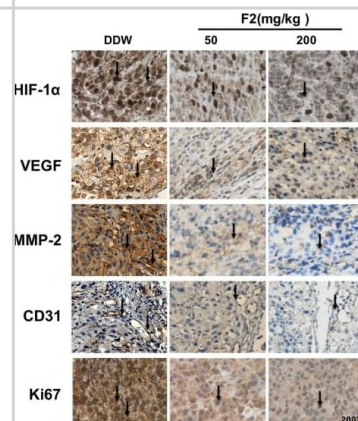
AAM inhibits hypoxia-induced VM formation involving HIF-1α and MMP2. The HCT-116 (A) and LoVo (B) cells were treated with different concentrations of AAM under hypoxia and normoxia; the protein expression of HIF-1α and MMP2 was detected by western blotting. (C, D) The mRNA expression of HIF-1α and MMP2 was measured by RT-PCR (*P < 0.05, **P < 0.01, ***P < 0.001).
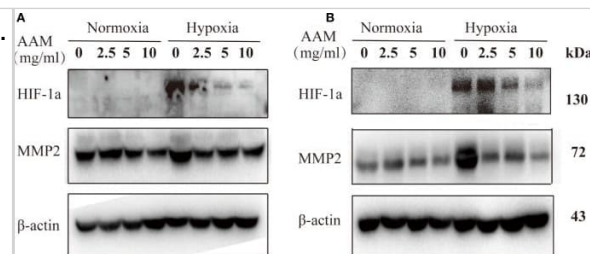
Immunohistochemical analysis of STC1 expression in esophageal cancer. (A) Normal squamous epithelium was negative for STC1 expression. (B) Normal gastric glands showed positive staining for STC1, which was used as a positive control. (C) Positive staining for STC1 in esophageal squamous cell carcinoma (ESCC). (D) Negative staining for STC1 in ESCC. (E) Positive staining for HIF-1α in ESCC. (F) Positive staining for p53 in ESCC. Magnification, ×100.
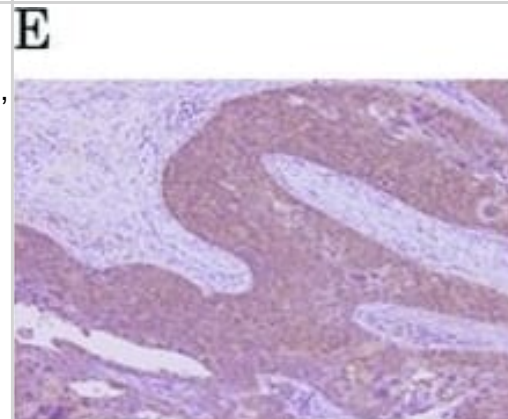
Publications

Q Lin, S Li, N Jiang, H Jin, X Shao, X Zhu, J Wu, M Zhang, Z Zhang, J Shen, W Zhou, L Gu, R Lu, Z Ni Inhibiting NLRP3 inflammasome attenuates apoptosis in contrast-induced acute kidney injury through the upregulation of HIF1A and BNIP3-mediated mitophagy Autophagy, 2020-12-19;0(0):1-16. 2020-12-19 [PMID: 33345685]

Bae YH, Joo H, Bae J et al. Brain injury induces HIF-1a-dependent transcriptional activation of LRRK2 that exacerbates brain damage. Cell Death Dis. 2018-11-12 [PMID: 30420654]

Nan Niu, Hui Li, Xiancai Du, Chan Wang, Junliang Li, Jihui Yang, Cheng Liu, Songhao Yang, Yazhou Zhu, Wei Zhao Effects of NRF-1 and PGC-1α cooperation on HIF-1α and rat cardiomyocyte apoptosis under hypoxia. Gene 2022-06-21 [PMID: 35569770]

RytkOnen K, Faux T, Mahmoudian M et al. Histone H3K4me3 breadth in hypoxia reveals endometrial core functions and stress adaptation linked to endometriosis iScience 2022-05-01 [PMID: 35494227]

Chen S, Wang Y, Xiong Y et al. Wild-type IDH1 inhibits the tumor growth through degrading HIF-alpha in renal cell ijbs.com 2021-01-01 [PMID: 33867843]

Reiji Ito, Elizabeth A Barnes, Xibing Che, Cristina M Alvira, David N Cornfield SM22α cell-specific HIF stabilization mitigates hyperoxia-induced neonatal lung injury. American journal of physiology. Lung cellular and molecular physiology 2022-08-02 [PMID: 35762602]

Manivannan Yegambaram, Xutong Sun, Alejandro Garcia Flores, Qing Lu, Jamie Soto, Jaime Richards, Saurabh Aggarwal, Ting Wang, Haiwei Gu, Jeffrey R. Fineman, Stephen M. Black, Venediktova Natalya Novel Relationship between Mitofusin 2-Mediated Mitochondrial Hyperfusion, Metabolic Remodeling, and Glycolysis in Pulmonary Arterial Endothelial Cells International Journal of Molecular Sciences 2023-12-15 [PMID: 38139362]

Romana Bohuslavova, Radka Cerychova, Frantisek Papousek, Veronika Olejnickova, Martin Bartos, Agnes Görlach, Frantisek Kolar, David Sedmera, Gregg L. Semenza, Gabriela Pavlinkova HIF-1α is required for development of the sympathetic nervous system Proceedings of the National Academy of Sciences of the United States of America 2019-07-02 [PMID: 31196952]

Han JH, Jang KW,Myung CS Garcinia cambogia attenuates adipogenesis by affecting CEBPB and SQSTM1/p62-mediated selective autophagic degradation of KLF3 through RPS6KA1 and STAT3 suppression Autophagy 2021-06-08 [PMID: 34101546]

Nathan K. P. Wong, Emma L. Solly, Richard Le, Victoria A. Nankivell, Jocelyne Mulangala, Peter J. Psaltis, Stephen J. Nicholls, Martin K. C. Ng, Christina A. Bursill, Joanne T. M. Tan, Riccardo Alessandro TRIM2 Selectively Regulates Inflammation-Driven Pathological Angiogenesis without Affecting Physiological Hypoxia-Mediated Angiogenesis International Journal of Molecular Sciences 2024-03-15 [PMID: 38542330]

Guo S, Geng X, Lee H, Ding Y Phenothiazine Inhibits Neuroinflammation and Inflammasome Activation Independent of Hypothermia After Ischemic Stroke Research Square 2021-07-07 [PMID: 34455546]

Nicholas D Nolan, Xuan Cui, Brian M Robbings, Aykut Demirkol, Kriti Pandey, Wen-Hsuan Wu, Hannah F Hu, Laura A Jenny, Chyuan-Sheng Lin, Daniel T Hass, Jianhai Du, James B Hurley, Stephen H Tsang CRISPR editing of anti-anemia drug target rescues independent preclinical models of retinitis pigmentosa. Cell reports. Medicine 2024-04-19 [PMID: 38518771]

More publications at

Procedures

Western Blot protocol for HIF-1 alpha Antibody (NB100-105)
General considerations for Western blot analysis of HIF-alpha proteins:

1. HIF-1alpha is largely undetectable in cells or tissues grown under normoxic conditions. It is stabilized only at O2 concentrations below 5% or with treatment using certain agents (CoCl2, DFO, etc.), therefore proper sample preparation is critical. We recommend lysing cells quickly and directly into the Laemmli sample buffer with DTT or BME.
2. Since stabilized HIF-1alpha translocates to the nucleus, using nuclear extracts is recommended for western blot analysis.
3. Positive and negative controls should always be run side by side in a Western blot to accurately identify the protein band upregulated in the hypoxic sample. (HeLa Hypoxic/Normoxic Cell Lysate: NBP2-36452; HeLa Hypoxic (CoCl2)/Normoxic Lysate: NBP2-36450)
4. To accurately compare treated and untreated samples and to ensure equal loading of samples the expression of a loading control should be evaluated. (alpha Tubulin Antibody (DM1A): NB100-690)
5. Unprocessed HIF-1alpha is ~95 kDa, while the fully post-translationally modified form is ~116 kDa, or larger.
6. HIF-1alpha may form a heterodimer with HIF-1beta (Duan, et al. Circulation. 2005; 111:2227-2232.). However, this is not typically seen under denaturing conditions.
7. Depending on the sample and treatment, a single band or a doublet may be present.
8. Please, note that NB100-105 as a monoclonal antibody hence it is much weaker then polyclonal antibodies and need very sensitive detection reagents (e.g. Supersignal West Pico Plus, or more sensitive one) and long time exposure to obtain WB image.

Western Blot Protocol

1. Load samples of treated and untreated cell lysates, 10-40 mg of total protein per lane on a 7.5%polyacrylamide gel (SDS-PAGE). Alternatively, gradient gels can be used for better resolution of lower molecular weight loading controls.
2. Resolve proteins by electrophoresis as required.
3. Transfer proteins to 0.45 mm PVDF membrane for 1 hour at 100V or equivalent.
4. Stain the blot using Ponceau S for 1-2 minutes to confirm efficient protein transfer onto the membrane.
5. Rinse the blot in distilled water to remove excess stain and mark the lanes and locations of molecular weight markers using a pencil.
6. Block the membrane using 5% non-fat dry milk in TBST (0.1% Tween) for 1 hour.
7. Dilute the mouse anti-HIF-1 alpha primary antibody (NB100-105) at 2ug/ml in blocking solution and incubate 1 hour at room temperature or overnight at 4C.
8. Wash the membrane 3X 5 min in TBST.
9. Incubate in the appropriate diluted mouse-IgG HRP-conjugated secondary antibody in blocking solution (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the membrane 3X 5 min in TBST.
11. Incubate with ECL detection reagent (Supersignal West Pico Plus, or more sensitive) for 5 min.
12. Image the blot. That may require up to 5min of exposure due to weak signal.

Immunohistochemistry-Paraffin protocol for HIF-1 alpha Antibody (NB100-105)
HIF-1 alpha Antibody (H1alpha67):
Immunohistochemistry Protocol:

1. Prepare tissue with formalin fixation and by embedding it in paraffin wax.
2. Make 4-mm sections and place on pre-cleaned and charged microscope slides.
3. Heat in a tissue-drying oven for 45 minutes @ 60 degrees Celcius.
4. Deparaffinize the tissues by wash drying the slides in 3 changes of xylene for 5 minutes each @ RT.
5. Rehydrate the tissues by washing the slides in 3 changes of 100% alcohol for 3 minutes each @ RT.
6. Wash the slides in 2 changes of 95% alcohol for 3 minutes each @ RT.
7. Wash the slides in 1 change of 80% alcohol for 3 minutes @ RT.
8. Rinse the slides in gentle running distilled water for 5 minutes @ RT.
9. Perform antigen retrieval by steaming the slides in 0.01M sodium citrate buffer (pH 6.0) @ 99-100 degrees Celcius for 20 minutes.
10. Remove the slides from the heat and let stand in buffer @ RT for 20 minutes.
11. Rinse the slides in 1X TBS-T for 1 minute @ RT.
Do not allow the tissues to dry at any time during the staining procedure
12. Begin the immunostaining by applying a universal protein block for 20 minutes @ RT.
13. Drain protein block from the slides and apply the diluted primary antibody for 45 minutes @ RT.
14. Rinse the slide in 1X TBS-T for 1 minute @ RT.
15. Apply a biotinylated anti-mouse (H+L) secondary for 30 minutes @ RT.
16. Rinse the slide in 1X TBS-T for 1 minute @ RT.
17. Apply an alkaline phosphatase streptavidin for 30 minutes @ RT.
18. Rinse the slide in 1X TBS-T for 1 minute @ RT.
19. Apply an alkaline phosphatase chromogen substrate for 30 minutes @ RT.
20. Rinse the slide in distilled water for 1 minute @ RT.
This method should only be used if the chromogen substrate is alcohol insoluble (ie: Vector Red, DAB)
21. Dehydrate the tissue by washing the slides in 2 changes of 80% alcohol for 1 minute each @ RT.
22. Wash the slides in 2 changes of 95% alcohol for 1 minute each @ RT.
23. Wash the slides in 3 changes of 100% alcohol for 1 minute each @ RT.
24. Wash the slides in 3 changes of xylene for 1 minute each @ RT.
25. Apply cover slip.

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Products Related to NB100-105

NBP2-36452	HeLa Hypoxic / Normoxic Cell Lysate
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP2-27231	Mouse IgG2b Isotype Control (MPC-11)

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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