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.

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**NB100-105**

**HIF-1 alpha Antibody (H1alpha67)**

### Product Information

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

### 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

**Applications**


**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. Simple Western reported by an internal validation. Separated by Size

### Images

<table>
<thead>
<tr>
<th>HIF-1 alpha induction by CoCl2 on Caki-1 cell lysate. WB image submitted by a verified customer review.</th>
</tr>
</thead>
<tbody>
<tr>
<td>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.</td>
</tr>
<tr>
<td>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.</td>
</tr>
</tbody>
</table>
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 (http://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 (http://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.
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 (http://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 (http://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.

Publications


Xie Y, Guo W, Shen X et al. A delayed ovulation of progestin-primed ovarian stimulation (PPOS) by downregulating the LHCGR/PGR pathway iScience 2023-08-18 [PMID: 37520702] (ICC/IF, Mouse)

Wu K, Li B, Ma Y et al. Nicotinamide mononucleotide attenuates HIF-1? activation and fibrosis in hypoxic adipose tissue via NAD+/SIRT1 axis Frontiers in endocrinology 2023-01-26 [PMID: 36777361] (IP, Mouse)

Guo J, Zhou Q, Huang W et al. CDCA8 promotes bladder cancer survival by stabilizing HIF1? expression under hypoxic Research Square 2023-05-03


Details:
ICC/IF 1:200, WB 1:1000

Qiu M, Li C, Cai Z et al. 3D Biomimetic Calcified Cartilaginous Callus that Induces Type H Vessels Formation and Osteoclastogenesis Advanced science (Weinheim, Baden-Wurttemberg, Germany) 2023-03-31 [PMID: 36999832] (IHC-P, Rat)


Ju Z, Hu Q, Sun W et al. CK-666 protects against ferroptosis and renal ischemia-reperfusion injury through a microfilament-independent mechanism Research Square 2023-04-25 (WB)


More publications at http://www.novusbio.com/NB100-105
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.
4. To accurately compare treated and untreated samples and to ensure equal loading of samples the expression of a loading control should be evaluated.
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 seeing 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): https://www.novusbio.com/products/hif-1-alpha-antibody-h1alpha67_nb100-105

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 Celsius.
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 Celsius 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.
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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