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

<table>
<thead>
<tr>
<th>Host</th>
<th>Mouse</th>
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<tbody>
<tr>
<td>Gene ID</td>
<td>3091</td>
</tr>
<tr>
<td>Gene Symbol</td>
<td>HIF1A</td>
</tr>
<tr>
<td>Species</td>
<td>Human, Mouse, Rat, Porcine, Bovine, Canine, Ferret, Monkey, Primate, Rabbit, Sheep, Xenopus</td>
</tr>
</tbody>
</table>

### Reactivity Notes

Xenopus reactivity was reported in scientific literature (PMID: 18303027). Please note that this antibody is reactive to Mouse and derived from the same host, Mouse. Additional Mouse on Mouse blocking steps may be required for IHC and ICC experiments. Please contact Technical Support for more information. Canine reactivity reported in scientific literature (PMID: 24153191, 29974500).

### Immunogen

A fusion protein containing amino acids 432 - 528 of human HIF-1 alpha [UniProt# Q16665].

## Product Application Details

### Applications

- Western Blot
- Chromatin Immunoprecipitation
- ELISA
- Flow Cytometry
- Gel Super Shift Assays
- Immunocytochemistry/Immunofluorescence
- Immunohistochemistry
- Immunohistochemistry-Frozen
- Immunohistochemistry-Paraffin
- Immunoprecipitation
- Ligand Activation
- Proximity Ligation Assay
- Tissue Culture Substratum
- Knockout Validated

### Recommended Dilutions

ChIP usage was reported in scientific literature. ELISA usage was reported in scientific literature (PMID: 20042684). Gel Super Shift Assays usage was reported in scientific literature (PMID: 22411794). Ligand Activation usage was reported in scientific literature (PMID: 26147748). 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. Knock Out Validation was reported in scientific literature (PMID: 26861754). Use in proximity ligation assay reported in scientific literature (PMID 27595394). We recommend the use of a highly sensitive ECL reagent, such as West Pico PLUS, for Western blot detection.

Images

Western Blot: HIF-1 alpha Antibody (H1alpha67) [NB100-105] - HIF-1 alpha induction by CoCl2 on Caki-1 cell lysate. Image from verified customer review.

Knockout Validated: HIF-1 alpha Antibody (H1alpha67) [NB100-105] - 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 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to nuclei.

Western Blot: HIF-1 alpha Antibody (H1alpha67) [NB100-105] - Analysis using the HRP conjugate of NB100-105. Detection of 50ug cobalt chloride induced COS-7 nuclear extracts (NB800-PC26) using NB100-105.
Western Blot: HIF-1 alpha Antibody (H1alpha67) [NB100-105] - Analysis of HIF-1 alpha in human hepatocytes from cancer patient using anti-HIF-1 alpha antibody. Image from verified customer review.

Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-105] - Staining of HIF1 alpha in human kidney. Renal tubular epithelium showed moderate membranous, cytoplasmic and nuclear staining, and glomeruli showed faint to moderate nuclear staining.

Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (H1alpha67) [NB100-105] - Analysis using the Biotin conjugate of NB100-105. Staining of human glioblastoma multiforme.

Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (H1alpha67) [NB100-105] - Expression of HIF-1 in intrestitial pig cells under 12% of O2 condition. Image from verified customer review.
Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (H1alpha67) [NB100-105] - HIF-1a was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line treated with DFOA using 1 ug/mL of mouse anti-HIF-1a monoclonal (NB100-105, Novus Biologicals). Cells were stained using donkey anti-rabbit and counterstained with DAPI (blue).

Flow Cytometry: HIF-1 alpha Antibody (H1alpha67) [NB100-105] - Analysis using the Alexa Fluor (R) 488 conjugate of NB100-105. Staining of HIF-1 alpha in H929 cells using HIF-1 alpha antibody. Image from verified customer review.

Flow Cytometry: HIF-1 alpha Antibody (H1alpha67) [NB100-105] - Analysis using the Alexa Fluor (R) 488 conjugate of NB100-105. Staining of HIF-1 alpha in multiple myeloma cells: H929 cells (0.5x10^6) were stained with Alexa Fluor 488 (R) conjugated HIF-1a antibody (NB100-105AF488). Image courtesy of Dr. Barbara Muz at Washington University in St. Louis School of Medicine.

Western Blot: HIF-1 alpha Antibody (H1alpha67) [NB100-105] - HeLa whole cell lysate. ECL solution 5 min, exposure 3-5 min. Image from verified customer review.
Western Blot: HIF-1 alpha Antibody (H1alpha67) [NB100-105] - Naive CD4 T cells from WT, VHL-deficient (Vhl KO), or HIF-1a-deficient (Hif1a 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-1a and Lamin B1 levels were analyzed by Western blot. Image from verified customer review.

**Publications**

Weaver J, Casellini C, Parson H, Vinik A. EXPRESSION OF HIF-1a IN A PANCREATIC DUCTAL ADENOCARCINOMA IN A PATIENT WITH NEWLY DIAGNOSED TYPE 2 DIABETES AACE Clinical Case Reports May 1 2018 12:00AM

Lanigan S, Corcoran AE, Wall A et al. Acute hypoxic exposure and prolyl-hydroxylase inhibition improves synaptic transmission recovery time from a subsequent hypoxic insult in rat hippocampus. Brain Res. Sep 20 2018 12:00AM [PMID: 30244114] (WB, Rat)


Cheng CC, Chi PL, Shen MC et al. Caffeic Acid Phenethyl Ester Rescues Pulmonary Arterial Hypertension through the Inhibition of AKT/ERK-Dependent PDGF/HIF-1alpha In Vitro and In Vivo Int J Mol Sci Mar 22 2019 12:00AM [PMID: 30909527] (WB, Rat)


Hicks KC, Patel TB. Sprouty 2 Protein Regulates Hypoxia-inducible Factor-alpha (HIF1-alpha) Protein Levels and Transcription of HIF1- alpha responsive Genes J. Biol. Chem. 2016 Aug 05 [PMID: 27281823] (IP, Human)


Fujimoto TN, Colbert LE, Molkentine JM, Baseler L. EGLN Inhibition Reduces Gastrointestinal Radiation Toxicity and Improves Survival in a Murine Model of Locally Advanced Pancreatic Cancer bioRxiv 2018 Jul 20 (WB, Mouse)

Zhang F, Li R, Yang Y et al. Specific Decrease in B-Cell-Derived Extracellular Vesicles Enhances Post-Chemotherapeutic CD8+ T Cell Responses Immunity Feb 6 2019 12:00AM [PMID: 30770248] (WB, Mouse)


More publications at [http://www.novusbio.com/NB100-105](http://www.novusbio.com/NB100-105)
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: NBP-36452; HeLa Hypoxic (CoCl2)/Normoxic Lysate: NBP-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 than 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)

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-rabbit IgG (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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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