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: 33  Publications: 745

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Updated 11/20/2018 v.20.1
### NB100-105
HIF-1 alpha Antibody (H1alpha67)

#### Product Information

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</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>
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<tr>
<td>Storage</td>
<td>Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td>Clonality</td>
<td>Monoclonal</td>
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<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>
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<tr>
<td>Buffer</td>
<td>PBS with 1% BSA</td>
</tr>
<tr>
<td>Target Molecular Weight</td>
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</table>

#### Product Description

**Host**
Mouse

**Gene ID**
3091

**Gene Symbol**
HIF1A

**Species**
Human, Mouse, Rat, Porcine, Bovine, Ferret, Monkey, Primate, Rabbit, Sheep, Xenopus

**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.

**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**

**Application Notes**
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).
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 ug/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 interstitial 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 µg/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] - Figure Legend: 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. This image was submitted via customer Review. (Mouse, WB)
Publications


Drareni K, Ballaire R, Barilla S et al. GPS2 Deficiency Triggers Maladaptive White Adipose Tissue Expansion in Obesity via HIF1A Activation. Cell Rep Sep 11 2018 12:00AM [PMID: 30208320] (WB, Mouse)

Jacobo-Estrada T, Cardenas-Gonzalez M, Santoyo-Sanchez MP et al. Intrauterine Exposure to Cadmium Reduces HIF-1 DNA-Binding Ability in Rat Fetal Kidneys. Toxics Sep 3 2018 12:00AM [PMID: 30177602] (WB, Rat)


Hayashi Y, Zhang Y, Yokota A et al. Pathobiologic Pseudohypoxia as a Putative Mechanism Underlying Myelodysplastic Syndromes Cancer Discov Aug 23 2018 12:00AM [PMID: 30139811] (ICC/IF, Mouse)


Tatli Dogan H, Kiran M, Bilgin B et al. Prognostic significance of the programmed death ligand 1 expression in clear cell renal cell carcinoma and correlation with the tumor microenvironment and hypoxia-inducible factor expression Diagn Pathol Aug 25 2018 12:00AM [PMID: 30144808] (IHC, Human)

Oe Yuji, Ko Mieko, Fushima Tomofumi et al. Hepatic dysfunction and thrombocytopenia induced by excess sFlt1 in mice lacking endothelial nitric oxide synthase. Scientific Reports 2018 [PMID: 29311569] (IHC, Human)

Toblli Jorge E, Cao Gabriel, Giani Jorge F et al. Markers of oxidative/nitrosative stress and inflammation in lung tissue of Rats exposed to different intravenous iron compounds. Drug Design, Development and Therapy 2017 [PMID: 28814833] (IHC, Rat)


More publications at http://www.novusbio.com/NB100-105
Procedures

Western Blot Protocol for HIF-1 alpha Antibody (NB100-105)

Western Blot Protocol Specific for HIF-1alpha 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.
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.

Western Blot Protocol

Materials
1x Laemmli Sample Buffer: 2% SDS, 2.5% 2-mercaptoethanol (bME), 25% glycerol, 0.01% bromophenol blue, 62.5 mM Tris HC, pH 6.8
1X Running Buffer: 25 mM Tris-base, 192 mM glycine, 0.1% SDS. Adjust to pH 8.3
1X Transfer buffer (wet): 25 mM Tris-base, 192 mM glycine, 20% methanol
1X TBS
TBST (1X TBS with 0.1% Tween-20)
Blocking solution: TBST with 5% non-fat dry milk
Mouse monoclonal anti-HIF-1 alpha primary antibody (NB100-105) in blocking solution (≈1-2 ug/mL)

Methods

Whole-Cell Lysates
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 TBS for 1 hour.
7. Dilute the mouse anti-HIF-1 alpha primary antibody (NB100-105) in blocking solution (1-2 ug/ml) and incubate 1 hour at room temperature or overnight at 4oC.
8. Wash the membrane 3X 10 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 10 min in TBST.
11. Apply the detection reagent of choice in accordance with the manufacturer’s instructions (e.g., ECL, ECL Plus).
12. Image the blot.
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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