Product Datasheet

HIF-1 alpha Antibody
NB100-105SS

Unit Size: 0.025 ml

Store at 4C. Do not freeze.

Reviews: 22    Publications: 462

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB100-105

Updated 6/15/2014 v.20.1
**NB100-105SS**  
HIF-1 alpha Antibody (H1alpha67)

### Product Information

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unit Size</strong></td>
<td>0.025 ml</td>
</tr>
<tr>
<td><strong>Concentration</strong></td>
<td>1.0 mg/ml</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>Store at 4C. Do not freeze.</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>0.02% 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</td>
</tr>
<tr>
<td><strong>Target Molecular Weight</strong></td>
<td>120 kDa</td>
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### Product Description

<table>
<thead>
<tr>
<th>Parameter</th>
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</tr>
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<tbody>
<tr>
<td><strong>Host</strong></td>
<td>Mouse</td>
</tr>
<tr>
<td><strong>Gene ID</strong></td>
<td>3091</td>
</tr>
<tr>
<td><strong>Gene Symbol</strong></td>
<td>HIF1A</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td>Human, Mouse, Rat, Bovine, Ferret, Primate, Porcine, Rabbit, Sheep, Xenopus, Yeast</td>
</tr>
<tr>
<td><strong>Species Reactivity</strong></td>
<td>Human, monkey, sheep, mouse, rat, rabbit, pig, bovine and ferret. Reactivity with Candida albicans reported by a customer review. Reactivity with Xenopus reported in the scientific literature (PMID: 18303027).</td>
</tr>
<tr>
<td><strong>Immunogen</strong></td>
<td>Fusion protein containing amino acids 432-528 of human HIF-1 alpha. [UniProt# Q16665]</td>
</tr>
<tr>
<td><strong>Notes</strong></td>
<td>There are reports that this antibody does not detect mouse in IHC-paraffin embedded tissue. It does work to detect mouse protein in Western blot.</td>
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### Product Application Details

<table>
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<th>Parameter</th>
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<tr>
<td><strong>Applications</strong></td>
<td>Western Blot, Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Gel Super Shift Assays, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation</td>
</tr>
</tbody>
</table>
**Application Notes**

This HIF-1 alpha (H1alpha67) antibody is useful for Chromatin Immunoprecipitation (PMID: 21871655), ELISA (PMID: 20042684), Flow Cytometry, Gel Super Shift Assays, Immunocytochemistry/Immunofluorescence, Immunohistochemistry on frozen and paraffin-embedded sections, Immunoprecipitation and Western Blot. 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. This antibody has been used to immunoprecipitate human HIF-1 alpha. For ChIP, refer to research papers with PubMed ID 16204079 and 21871655. This product has been cited for Gel Super Shift Assays in PubMed ID 22411794.

**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.

Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (H1alpha67) [NB100-105] - Expression of HIF-1 in intresititial pig cells under 12% of 02 condition. 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.
Flow Cytometry: HIF-1 alpha Antibody (H1alpha67) [NB100-105] - Analysis of HIF-1 alpha in multiple myeloma cells: H929 cells (0.5x10^6) were stained with HIF-1a antibody (NB100-105) conjugated with Alexa Fluor (R) 488. Image courtesy of Dr. Barbara Muz at Washington University in St. Louis School of Medicine.

Western Blot: HIF-1 alpha Antibody (H1alpha67) [NB100-105] - Analysis of 50ug cobalt chloride (CoCl2) induced COS-7 nuclear extracts (NB800-PC26).

Publications


Details: HIF1 alpha antibody used for WB on lysates of Duba+/+ and Duba-/- mouse CD4+ T(H)17 cells stimulated with anti-CD3 and anti-CD28 antibodies in the presence of TGF-b and IL-6 (Figure 7).


Details: HIF1 alpha antibody used for WB in experiments involving human colon carcinoma HCT116 and HT29 cells, human renal cancer UMRC2 cells and UMRC2/VHL cells (stably transfected with VHL)


Details: HIF1 alpha antibody used for IHC-P on sections of orthotopic mouse model with U87-LucNeo cells subjected to RNAi mediated knock down HIF-1 alpha in vivo
<table>
<thead>
<tr>
<th>Authors</th>
<th>Title</th>
<th>Journal</th>
<th>Publication Date [PMID: DOI]</th>
<th>Species</th>
<th>WB Details</th>
</tr>
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<tbody>
<tr>
<td>Rahman Su, Lee Ms, Baek Jh et al.</td>
<td>The Prolyl Hydroxylase Inhibitor Dimethylxallylglycine Enhances Dentin Sialophosphoprotein Expression through VEGF-Induced Runx2 Stabilization</td>
<td>PLoS OnE</td>
<td>2014 Nov 05 [PMID: 25369078]</td>
<td>Rat</td>
<td>(WB, Rat)</td>
</tr>
<tr>
<td>Assar M, Sanchez-Puelles Jm, Royo I et al.</td>
<td>FM19G11 reverses endothelial dysfunction in rat and human arteries through stimulation of the PI3K/Akt/enOS pathway, independently of mTOR/HIF-1Alpha activation</td>
<td>Br. J. Pharmacol.</td>
<td>2014 Nov 03 [PMID: 25363469]</td>
<td>Rat</td>
<td>(WB, Rat)</td>
</tr>
</tbody>
</table>

More publications at [http://www.novusbio.com/NB100-105](http://www.novusbio.com/NB100-105)
Procedures

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

HIF-1 alpha Western Blot General Information:

1. The HIF proteins are among the most rapidly degrading proteins ever studied. Upon cellular re-oxygenation it can be completely degraded in less than 1 minute. Therefore, it is critical to prep only a few plates/dishes/flasks of cells at a time and to immediately place the cells into ice cold buffers and perform the whole protein prep on ice.

2. HIF-1 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.) so proper sample preparation is critical.

3. Upon stabilization HIF-1 translocates to the nucleus. The best western blots (cleanest) are always done using nuclear extracts. It is possible to detect HIF-1 in whole cell extracts, but they tend to be much dirtier and the staining is much weaker.

4. Finally, we recommend that a positive/negative control always be run side by side so that it is possible to discern which band is upregulated in the hypoxic sample. Unprocessed HIF-1 is ~95 kDa while the fully post-translationally modified form is ~116 kDa, or larger. Additionally, HIF-1 alpha may form a heterodimer with HIF-1 beta (Duan, et al. Circulation. 2005;111:2227-2232.). Depending on the sample, treatment, etc. you may see either a band or a doublet.

"EPO transcription can be activated by exposure of Hep3B cells to either hypoxia or cobalt chloride (7). HIF-1 binding activity was induced after 1 h and was maximal after 4-h treatment of Hep3B cells with 75, M cobalt chloride (Fig. 2A), which is similar to the kinetics of HIF-1 induction by hypoxia (data not shown). Exposure of HeLa cells to cobalt chloride for 4 h also induced HIF-1 activity. In contrast to hypoxia, which induced a doublet band corresponding to HIF-1 in EMSAs, cobalt chloride induced a single band of HIF-1 activity in both Hep3B and HeLa cells (compare Figs. 1A and 2A). We have not determined the basis for this reproducible difference in response to stimulation by hypoxia as compared to cobalt chloride" (Wang G, et al. (1993) PNAS 90, 4304-4308.).

Thus, it is critical to be able to look at upregulation compared to the control.

Western Blot Protocol 1 (used to produce the image on the datasheet)

1. Perform SDS-PAGE (3-8%) on samples to be analyzed, loading 40ug of total protein per lane (COS-7 treated and untreated lysates.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. 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% non-fat dry milk in TBS for 1 hour.
6. Dilute the mouse anti-HIF-1 alpha primary antibody (NB 100-105) in blocking buffer and incubate 2 hours at room temperature.
7. Wash the membrane in water for 5 minutes and apply the diluted mouse-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) and incubate 1 hour at room temperature.
8. Wash the blot in TBS containing 0.05-0.1% Tween-20 for 10-20 minutes.
9. Wash the blot in type I water for an additional 10-20 minutes (this step can be repeated as required to reduce background.
10. Apply the detection reagent of choice in accordance with the manufacturers instructions (Amersham ECL is the standard reagent used at Novus Biologicals).

Note: Tween-20 can be added to the blocking buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

Western Blot Procedure 2
1) Resolve aliquots (25-30 ug) of induced nuclear protein extracts on a Tris-HCl gel.
2) Transfer to nitrocellulose membranes in 20 mM Tris-HCL (pH 8.0)/150 mM glycine/20% (vol/vol) methanol
3) Block membranes for 1 hour with 1X western wash buffer containing 5% non-fat dry milk (NFDM).
4) Incubate membranes overnight at 4C in NB 100-105 diluted 1:500 in 1X western wash/5% NFDM.
5) Wash with 1X western wash for 35 minutes at RT (1 X 15 minutes, 2 X 10 minutes).
6) Incubate membranes with 1:2,000 dilution of HRP conjugated anti-mouse IgG for 1 hour (RT) in 1X western wash/5% NFDM
7) Wash with 1X western wash for 35 minutes at RT (1 X 15 minutes, 2 X 10 minutes).
8) Drain membrane and place on saran wrap
9) Using Amersham ECL Kit, mix equal volumes of two reagents. Pour over membrane (protein side facing up). Let solution sit on membrane for 15-20 seconds.
10) Drain membrane and place on new saran wrap.
11) Wrap up membrane and expose to film.

Notes: If hypoxia treatment is not hypoxic enough (less than 2% oxygen to get an induction), signal will be absent. Also, if the harvest time is too slow or there are not enough protease inhibitors, etc., the induced protein will be rapidly lost as HIF-1alpha has a very short half-life.

**Immunohistochemistry Procedure (NB100-105)**

Immunohistochemistry Procedure
1. If not previously done, bake sections at 60C for 30 minutes.
2. Hydrate sections through the following series.
   A. 3 X 5 minutes xylenes
   B. 3 X 5 minutes 100% EtOH
   C. 2 minutes 95% EtOH
   D. 2 minutes 70% EtOH
   E. 1 minute 50% EtOH
   F. 1 minute ddH2O
   G. 1 minute TBS
3. If dry sections are needed, circle sample with wax pencil.
4. Antigen unmasking was performed by microwaving in 0.1M sodium citrate (pH 6.0) for 2 X 5 minutes at power level 7. Cool for 15 minutes.
5. Rinse slides with TBS.
6. Quench slides in 0.3% hydrogen peroxide in MeOH (0.5 ml 30% stock in 50 ml MeOH) for 25 minutes.
7. Wash 2 X 5 minutes with TBS.
8. Block sections with 10% serum (from the host species of the secondary antibody) in TBS for 30 minutes.
9. Incubate NB 100-105 with sections overnight at 4C at a 1:50 dilution in 10% goat serum.
10. The following day, allow sections to sit at RT for 30 minutes.
11. Wash sections 3 X 5 minutes in TBS, followed by incubation with the secondary antibody diluted 1:100 in 10% goat serum for 30 minutes at RT.
12. After 15 minutes of incubation, make up ABC solution and allow to sit for 30 minutes.
13. Wash slides 3 X 5 minutes in TBS.
14. Block with ABC solution in TBS.
15. Make DAB solution.
16. Incubate with fresh DAB solution until signal develops, then place in ddH2O. Dehydrate sections through graded alcohols to xylenes (reverse hydration steps) and coverslip with permount.
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 guarantee, please visit www.novusbio.com/guarantee.