Product Datasheet

HIF-1 alpha Antibody
NB100-105SS

Unit Size: 0.025 ml
Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

Reviews: 23  Publications: 481

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

Updated 6/15/2014 v.20.1
### Product Information

<table>
<thead>
<tr>
<th>Feature</th>
<th>Detail</th>
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<tbody>
<tr>
<td>Unit Size</td>
<td>0.025 ml</td>
</tr>
<tr>
<td>Concentration</td>
<td>1.0 mg/ml</td>
</tr>
<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>
</tr>
<tr>
<td>Clone</td>
<td>H1alpha67</td>
</tr>
<tr>
<td>Preservative</td>
<td>0.05% Sodium Azide</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG2b</td>
</tr>
<tr>
<td>Purity</td>
<td>Protein G purified</td>
</tr>
<tr>
<td>Buffer</td>
<td>PBS with 1% BSA</td>
</tr>
<tr>
<td>Target Molecular Weight</td>
<td>120 kDa</td>
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</table>

### Product Description

<table>
<thead>
<tr>
<th>Host</th>
<th>Mouse</th>
</tr>
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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, Bovine, Ferret, Primate, Porcine, Rabbit, Sheep, Xenopus, Yeast</td>
</tr>
<tr>
<td>Species Reactivity</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>
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<tr>
<td>Immunogen</td>
<td>Fusion protein containing amino acids 432-528 of human HIF-1 alpha. [UniProt# Q16665]</td>
</tr>
<tr>
<td>Notes</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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<tr>
<th>Applications</th>
<th>Western Blot, Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Gel Super Shift Assays, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation</th>
</tr>
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</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.

![Western Blot Image](image-url)

**Immunocytochemistry/Immunofluorescence:** HIF-1 alpha Antibody (H1alpha67) [NB100-105] - Expression of HIF-1 in interstitial pig cells under 12% of 02 condition. Image from verified customer review.

![Immunocytochemistry Image](image-url)

**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 Image](image-url)
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).
<table>
<thead>
<tr>
<th>Title</th>
<th>Authors</th>
<th>Journal/Conference/Book</th>
<th>Publication Date</th>
<th>PMID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic opioids regulate KATP channel subunit Kir6.2 and carbonic anhydrase I and II expression in rat adrenal chromaffin cells via HIF-2alpha and protein kinase A.</td>
<td>Salman Shaima, Holloway Alison C, Nurse Colin A.</td>
<td>Am J Physiol Cell Physiol.</td>
<td>2014 Aug 01</td>
<td>24898587 [Rat]</td>
</tr>
<tr>
<td>Robust rat pulmonary radioprotection by a lipophilic Mn N-alkylpyridylporphyrin, MnTnHex-2-PyP(5+).</td>
<td>Gauter-Fleckenstein Benjamin, Reboucas Julio S, Fleckenstein Katharina et al.</td>
<td>Redox Biol.</td>
<td>2014 Jan 09</td>
<td>24624330 [IHC, Rat]</td>
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<tr>
<td>Enhanced Hypoxia-Inducible Factor (HIF)-1 alpha Stability Induced by 5-Hydroxymethyl-2-Furfural (5-HMF) Contributes to Protection against Hypoxia</td>
<td>He YL, Li MM, Wu LY et al.</td>
<td>Mol. Med.</td>
<td>2015 Mar 27</td>
<td>25333920 [WB, ICC/IF, Mouse, Rat]</td>
</tr>
<tr>
<td>Partial deficiency of HIF-1alpha stimulates pathological cardiac changes in streptozotocin-induced diabetic mice.</td>
<td>Bohuslavova Romana, Kolar Frantisek, Sedmera David et al.</td>
<td>BMC Endocr Disord.</td>
<td>2014 Feb 06</td>
<td>24502509 [WB, Mouse]</td>
</tr>
<tr>
<td>Overexpression of hypoxia-inducible factor-1alpha is a predictor of poor prognosis in cervical cancer: a clinicopathologic study and a meta-analysis.</td>
<td>Huang Miaoling, Chen Qing, Xiao Jianpeng et al.</td>
<td>Int J Gynecol Cancer.</td>
<td>2014 Jul 01</td>
<td>24978711 [Human]</td>
</tr>
<tr>
<td>DMOG ameliorates IFN-gamma-induced intestinal barrier dysfunction by suppressing PHD2-dependent HIF-1alpha degradation.</td>
<td>Wang Wen-Sheng, Liang Hong-Yin, Cai Yu-Jiao, Yang Hua.</td>
<td>J Interferon Cytokine Res.</td>
<td>2014 Jan 01</td>
<td>24010824 [Human]</td>
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</table>

More publications at [http://www.novusbio.com/NB100-105](http://www.novusbio.com/NB100-105)
# 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 O\textsubscript{2} concentrations below 5% or with treatment using certain agents (CoCl\textsubscript{2}, 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\textsubscript{1} is \textasciitilde 95 kDa while the fully post-translationally modified form is \textasciitilde 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 \textmu 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 new 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.
12) Develop accordingly.

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.