Product Datasheet

HIF-1 alpha Antibody
NB100-105SS

Unit Size: 0.025 ml

Store at 4C. Do not freeze.

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NB100-105SS

HIF-1 alpha Antibody (H1alpha67)

Product Information	
Unit Size	0.025 ml
Concentration	1.0 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Monoclonal
Clone	H1alpha67
Preservative	0.02% Sodium Azide
Isotype	IgG2b
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	120 kDa

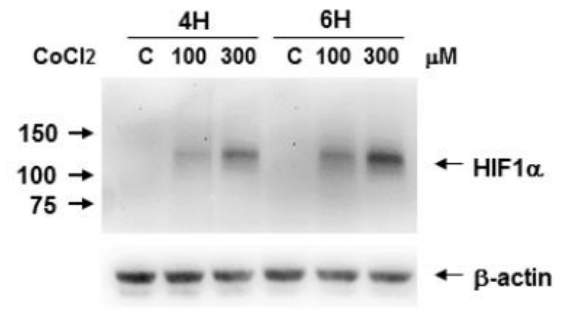
Product Description	
Host	Mouse
Gene ID	3091
Gene Symbol	HIF1A
Species	Human, Mouse, Rat, Bovine, Ferret, Primate, Porcine, Rabbit, Sheep, Xenopus, Yeast
Species Reactivity	Human, monkey, sheep, mouse, rat, rabbit, pig, bovine and ferret. Reactivity with Candida albicans reported by a customer review. Reactivity with Xenopus reported in the scientific literature (PMID: 18303027).
Immunogen	Fusion protein containing amino acids 432-528 of human HIF-1 alpha. [UniProt# Q16665]
Notes	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.

Product Application Details	
Applications	Western Blot, Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Gel Super Shift Assays, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Chromatin Immunoprecipitation 1:10-1:500, ELISA, Flow Cytometry, Gel Super Shift Assays 1:1-1:100, Immunocytochemistry/Immunofluorescence 1:50, Immunohistochemistry 1:20-1:50, Immunohistochemistry-Frozen 1:20-1:50, Immunohistochemistry-Paraffin 1:20-1:50, Immunoprecipitation 1:10-1:500, Western Blot 1:500

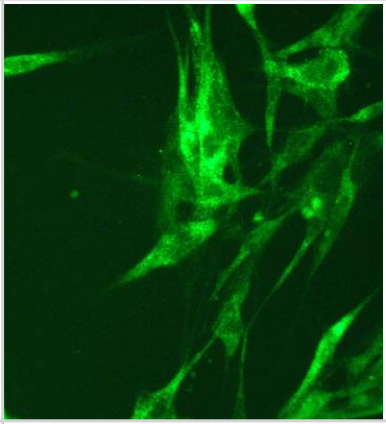
Application Notes	This HIF-1 alpha (H1alpha67) antibody is useful for Chromatin Immunoprecipitation (PMID: 21871655), ELISA (PMID: 20042684), Flow Cytometry, Gel Super Shift Assays, Immunocytochemistry/Immunofluorescence, Immunohistochemistry on frozen and paraffin-embedded sections, Immunoprecipitation 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

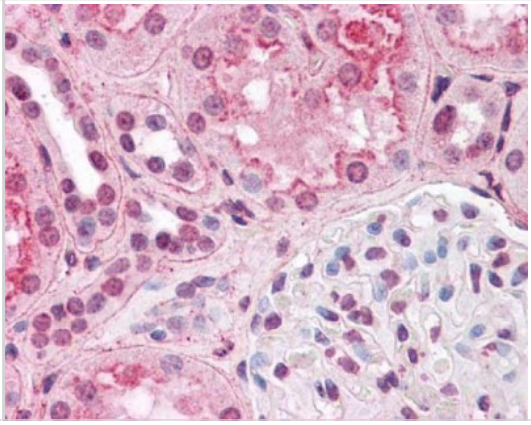
Western Blot: HIF-1 alpha Antibody (H1alpha67) [NB100-105] - HIF-1 alpha induction by CoCl2 on Caki-1 cell lysate. Image from verified customer review.

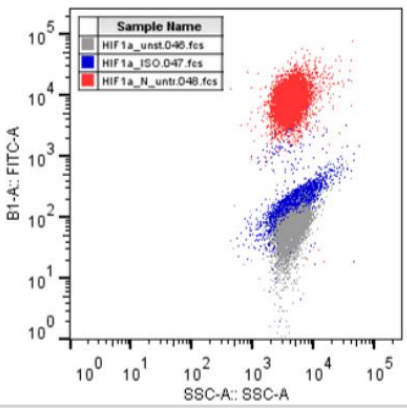
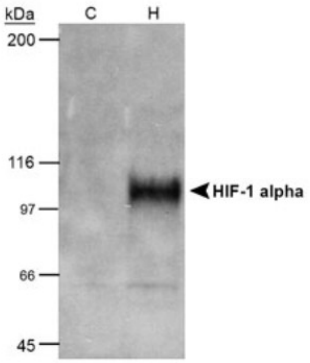


Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (H1alpha67) [NB100-105] - Expression of HIF-1 in intresititial pig cells under 12% of 02 condition. Image from verified customer review.



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

Queisser MA, Dada LA, Deiss-Yehiely N. HOIL-1L Functions as the PKC-zeta Ubiquitin Ligase to Promote Lung Tumor Growth. Am. J. Respir. Crit. Care Med. 2014 Aug 13 [PMID: 25118570]

Hung YH, Chang SH, Huang CT et al. Inhibitor of Differentiation-1 and Hypoxia-Inducible Factor-1 Mediate Sonic Hedgehog Induction by Amyloid Beta-Peptide in Rat Cortical Neurons. Mol. Neurobiol. 2014 Dec 15 [PMID: 25502463] (ChIP, Rat)

Rutz S, Kayagaki N, Phung QT et al. Deubiquitinase DUBA is a post-translational brake on interleukin-17 production in T cells. Nature. 2014 Dec 03 [PMID: 25470037] (WB, Mouse)

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).

Jeong S, Park H, Hong S et al. Lipophilic modification enhances anti-colitic properties of rosmarinic acid by potentiating its HIF-prolyl hydroxylases inhibitory activity. Eur. J. Pharmacol. 2014 Dec 04 [PMID: 25483211] (WB, Human)

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)

Gillespie DL, Aguirre MT, Ravichandran S et al. RNA interference targeting hypoxia-inducible factor 1alpha via a novel multifunctional surfactant attenuates glioma growth in an intracranial mouse model. J. Neurosurg. 2014 Nov 25 [PMID: 25423275] (IHC-P, Human)

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

Wu X, He L, Chen F et al. Impaired autophagy contributes to adverse cardiac remodeling in acute myocardial infarction. PLoS OnE. 2014 Nov 21 [PMID: 25409294] (WB, Mouse)

Sato Y, Miyauchi Y, Yoshida S et al. The Vitamin D Analogue ED71 but not 1,25(OH)2D3 Targets HIF1Alpha Protein in Osteoclasts PLoS OnE et al. 2014 Nov 07 [PMID: 25375896] (WB, Mouse)

Marhold M, Tomasich E, El-Gazzar A et al. HIF-1alpha Regulates mTOR Signaling and Viability of Prostate Cancer Stem Cells. Mol. Cancer Res. 2014 Oct 27 [PMID: 25349289] (WB, IP, Human, Mouse)

Yoshikawa N, Shimizu N, Ojima H et al. Down-regulation of hypoxia-inducible factor-1 alpha and vascular endothelial growth factor by HEXIM1 attenuates myocardial angiogenesis in hypoxic mice. Biochem. Biophys. Res. Commun. 2014 Oct 06 [PMID: 25301555] (WB, Human)

Rahman Su, Lee Ms, Baek Jh et al. The Prolyl Hydroxylase Inhibitor Dimethyloxalylglycine Enhances Dentin Sialophoshoprotein Expression through VEGF-Induced Runx2 Stabilization PLoS OnE et al. 2014 Nov 05 [PMID: 25369078] (WB, Rat)

Details:
HIF1 alpha antibody used for WB on the whole cell lysates of MDPC-23 cells (a rat odontoblast-like cell line) incubated for 24 h in the presence or absence of DMOG 0-1 mM concentration (Figure 1B).

Assar Me, Sanchez-Puelles Jm, Royo I et al. FM19G11 reverses endothelial dysfunction in rat and human arteries through stimulation of the PI3K/Akt/enOS pathway, independently of mTOR/HIF-1Alpha activation Br. J. Pharmacol. 2014 Nov 03 [PMID: 25363469] (WB, Rat)

Details:
HIF-1 alpha antibody used for WB on lysates of ex vivo FM19G11 treated aortic and mesenteric arteries from control and insulin-resistant rats (Figure 6).

Funk K, Scheerer N, Verhaegh R et al. Severe blunt muscle trauma in rats: only marginal hypoxia in the injured area PLoS OnE et al. 2014 Nov 01 [PMID: 25360779] (IHC-P, Rat)

Details:
HIF1 alpha antibody used for IHC-P on right Musculus gastrocnemius tissue section of male Wistar rats subjected to severe blunt muscle trauma with standardized weight-drop device - paraformaldehyde-fixed-paraffin-embedded sections, primary antibody dilution of 1:10000, catalyzed signal amplification system from DAKO, staining development with DAB-Hematoxylin (Figure 5).

More publications at 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 HIF1 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.
12) Develop accordingly. 10X Western wash 24.2g Tris 80g NaCl Tween-20 to 1% Ph 7.6 and QS to 4L Stripping buffer 100 mM BME 2% SDS 62.5 mM Tris (pH 6.7) Incubate membrane for 30 minutes at 56C
13) Wash membrane for 15 minutes with several changes of 1X western wash.

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.
Nuclear Extract Preparation Reference: Wang and Semenza. Purification and Characterization of Hypoxia-Inducible Factor. Journal of Biological Chemistry. 270(3): 1230-1237, 1995

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.

Novus Biologicals USA
8100 Southpark Way, A-8
Littleton, CO 80120
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
novus@novusbio.com

Novus Biologicals Canada
461 North Service Road West, Unit B37
Oakville, ON L6M 2V5
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada@novusbio.com

Novus Biologicals Europe
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: technical@novusbio.com
Orders: orders@novusbio.com
General: novus@novusbio.com

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.