Product Datasheet

HIF-1 alpha Antibody
NB100-449

Unit Size: 0.1 ml

Store at 4C. Do not freeze.

Reviews: 27  Publications: 212

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Updated 9/11/2019 v.20.1

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### Product Information

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Unit Size</td>
<td>0.1 ml</td>
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<tr>
<td>Concentration</td>
<td>0.2 mg/ml</td>
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<tr>
<td>Storage</td>
<td>Store at 4C. Do not freeze.</td>
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<tr>
<td>Clonality</td>
<td>Polyclonal</td>
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<tr>
<td>Preservative</td>
<td>0.09% Sodium Azide</td>
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<tr>
<td>Isotype</td>
<td>IgG</td>
</tr>
<tr>
<td>Purity</td>
<td>Immunogen affinity purified</td>
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<tr>
<td>Buffer</td>
<td>TBS and 0.1% BSA</td>
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<tr>
<td>Target Molecular Weight</td>
<td>93 kDa</td>
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</tbody>
</table>

### Product Description

**Host**
- Rabbit

**Gene ID**
- 3091

**Gene Symbol**
- HIF1A

**Species**
- Human, Mouse, Rat, Canine, Chicken, Goat, Monkey, Primate

**Reactivity Notes**
- Monkey (COS-7) and Rat reactivities were reported in customer review feedback. Detection of HIF1 alpha in both Mouse and Human tissue by IHC. Chicken reactivity was reported in scientific literature (PMID: 25632022). Canine reactivity reported in scientific literature (PMID: 28701694). Reactivity with Goat is reported in PMID: 21599540. Immunogen sequence is 100% to Panda, Orangutan, Rhesus Monkey, Gorilla, Chimpanzee, Grass Carp, Northern Pike, Atlantic Code, Duckbill Platypus, and Gansu Zokor.

**Immunogen**
- The immunogen recognized by this HIF-1 alpha Antibody maps to a region between residues 775 and the C-terminus (residue 826) of human hypoxia-inducible factor 1 [UniProt# Q16665].

### Product Application Details

**Applications**
- Western Blot, Simple Western, Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Immunocytochemistry, Immunofluorescence, Knockout Validated

**Recommended Dilutions**
- Western Blot, Simple Western 1:200, Chromatin Immunoprecipitation 1:10 - 1:500, Flow Cytometry 0.125 ug per 1 million cells in a 150 mcl reaction, ELISA 1:100-1:2000, Immunohistochemistry, Immunocytochemistry/Immunofluorescence 1:10-1:500, Immunoprecipitation 2-5 ug/mg lysate, Immunohistochemistry-Paraffin 1:50-1:200, Immunohistochemistry-Frozen 1:50-1:200, Immunofluorescence, Immunocytochemistry, Knockout Validated

**Application Notes**
- ChIP usage was reported in scientific literature (PMID: 25557133). In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. For IHC-P, Tris-EDTA pH 9.0 buffer is recommended for the heat induced epitope retrieval. ELISA (PMID: 17556599 and 16966370).
Western Blot: HIF-1 alpha Antibody [NB100-449] - HIF-1 alpha induction on Caki-1 cell lysate using CoCl2. Image from verified customer review.

Western Blot: HIF-1 alpha Antibody [NB100-449] - Detection of Human HIF-1 alpha by Western Blot and Immunoprecipitation. Samples: Whole cell lysate (5, 15 and 50 μg for WB; 1 mg for IP, 20% of IP loaded) from HeLa cells that were either treated with cobalt chloride (+; 200 μM) or mock treated (-). Antibodies: Affinity purified rabbit anti-HIF1 alpha antibody used for WB at 0.1 μg/mL (A) and 1 μg/mL (B) and used for IP at 3 μg/mg lysate. HIF-1 alpha was also immunoprecipitated by a previous lot of this antibody. Detection: Chemiluminescence with exposure times of 30 seconds (A) and 10 seconds (B).

Simple Western: HIF-1 alpha Antibody [NB100-449] - Simple Western lane view shows a specific band for HIF-1 alpha in 0.5 mg/ml of hypoxic HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

Immunofluorescence: HIF-1 alpha Antibody [NB100-449] - Murine primary bone marrow derived macrophages stained with HIF1-alpha antibody (red). Nuclei were counterstained with DAPI (blue). Image from verified customer review.

Flow Cytometry: HIF-1 alpha Antibody [NB100-449] - HeLa cells were treated for 15 hrs with 200 uM CoCl2, fixed in PFA, and permeabilized in 90% MeOH. 1 X 10^6 cells were stained with 0.125 ug anti-HIF-1 alpha and secondary FITC-conjugated goat anti-rabbit (in a 150 uL reaction). Black: treated, anti-KLH control IgG; Red: untreated, anti-HIF-1 alpha; Blue: treated, anti-HIF-1 alpha.

Western Blot: HIF-1 alpha Antibody [NB100-449] - Detection of human HIF1 alpha by western blot. Samples: Whole cell lysate (15 and 50 ug) from HEK293T cells that were either treated with 200 uM cobalt chloride (+) or mock treated (-). Antibodies: Affinity purified rabbit anti-HIF1 alpha antibody NB100-449 used for WB at 0.1 ug/ml. Detection: Chemiluminescence with exposure times of 3 minutes.

Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody [NB100-449] - Formaldehyde-fixed asynchronous HeLa cells.
Western Blot: HIF-1 alpha Antibody [NB100-449] - Analysis of HIF-1 alpha in human myeloma cell lysate using anti-HIF-1 alpha. Cells were untreated or treated with IGF-1, IL-6 or CoCl2. Image from verified customer review.

Western Blot: HIF-1 alpha Antibody [NB100-449] - Detection of HIF-1 alpha in a hypoxic sample. Lane 1: CoCl2 treated Cos-7 nuclear extract (hypoxic). Lane 2: Untreated Cos-7 nuclear extract (normoxic).

Western Blot: HIF-1 alpha Antibody [NB100-449] - Detection of mouse HIF-1 alpha on hypoxia treated MEFs

Western Blot: HIF-1 alpha Antibody [NB100-449] - Samples: Whole cell lysate (5, 15 and 50 ug) from HeLa cells that were treated with cobalt chloride (+; 200 uM) or mock treated (-). Antibodies: Affinity purified rabbit anti-HIF1 alpha antibody NB100-449 used for WB at 0.1 ug/ml. Detection: Chemiluminescence with exposure times of 30 seconds.
Western Blot: HIF-1 alpha Antibody [NB100-449] - BMDM were seeded at 0.5x10^6 overnight. Cells were treated with 10 ng/ml LPS for 24 hrs. Image from verified customer review.

Western Blot: HIF-1 alpha Antibody [NB100-449] - Analysis of total protein using anti-HIF-1 alpha, -CAIX, -ISCU antibodies and VDACs antibody (PMID: 29596470).

Western Blot: HIF-1 alpha Antibody [NB100-449] - Analysis of total protein using anti-HIF-1 alpha, -ISCU, -FXN antibodies. CoCl2 was used to treat or not HeLa cells for 2 days (PMID: 29596470).

Immunohistochemistry: HIF-1 alpha Antibody [NB100-449] - Mouse Brain, Neurons 40X
Immunoprecipitation: HIF-1 alpha Antibody [NB100-449] - HIF-1 alpha analysis in HEK293 cells. Image from verified customer review.

**Immunoprecipitation: HIF-1 alpha Antibody [NB100-449]**

Detection of human HIF1 alpha by western blot of immunoprecipitates. Samples: Whole cell lysate (1 mg for IP, 20% of IP loaded) from HEK293T cells that were treated with 200 uM cobalt chloride. Antibodies: Affinity purified rabbit anti-HIF1 alpha antibody NB100-449 used for WB at 1 ug/ml and for IP at 6 ug/mg lysate. Detection: Chemiluminescence with exposure times of 3 minutes.

**Table: DFO (hr)**

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<td>24</td>
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**Image:** 
- α-HIF1α

**Image:**
- IP/WB
- HIF1-alpha
- + + NB100-449
- + + CuCl2 Treatment
- - - Ctrl IgG
- + + IP

**MW (kDa)**
- 460
- 286
- 171
- 117
- 71
- 55
- 41
- 31
<table>
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<tr>
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<tr>
<td>Huang R, Hayashi Y, Yan X, et al. HIF1A is a critical downstream mediator for hemophagocytic lymphohistiocytosis. Haematologica 2017 Aug 31 [PMID: 28860338] (WB, Mouse)</td>
</tr>
<tr>
<td>Leinhos L Myofibroblast differentiation in hypoxia: a novel role for ArhGAP29 Thesis (WB)</td>
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<tr>
<td>Chowdhury D, Alrefai H, Landero Figueroa JA et al. Metallothionein 3 Controls the Phenotype and Metabolic Programming of Alternatively Activated Macrophages Cell Rep Jun 25 2019 12:00AM [PMID: 31242420] (ICC/IF, Mouse)</td>
</tr>
<tr>
<td>Chen Y, Zhao B, Zhu Y et al. HIF-1-VEGF-Notch mediates angiogenesis in temporomandibular joint osteoarthritis Am J Transl Res. May 15 2019 12:00AM [PMID: 31217867] (IHC, WB, Rat)</td>
</tr>
<tr>
<td>Bechmann N, Poser I, Seifert V et al. Dendritic Cell-Derived TSLP Negatively Regulates HIF-1 alpha and IL-1 beta During Dectin-1 Signaling Front Immunol May 8 2019 12:00AM [PMID: 31139177] (WB, Human)</td>
</tr>
<tr>
<td>Duan LJ, Fong GH. Developmental vascular pruning in neonatal mouse retinas is programmed in the astrocytic oxygen sensing mechanism Development Mar 25 2019 12:00AM [PMID: 30910827] (WB, Mouse)</td>
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</table>

More publications at [http://www.novusbio.com/NB100-449](http://www.novusbio.com/NB100-449)
Procedures

Immunohistochemistry Protocols (NB100-449)

IHC - Frozen

7 um mouse frozen sections were used.
Detection system: Vectors Anti-Rabbit Ig ImmPRESS Reagent Kit (cat # MP-7401)

1. Fix in ice cold acetone
2. Block for one hour at room temp. The block is provided by the vector kit; it is 2.5% horse serum.
3. Use NB 100-449 at a 1:100 dilution in PBS and incubate overnight in the fridge.
4. Perform a 15 min peroxidase block and incubated with the ImmPress anti-rabbit for 30 mins at RT.
5. Use DAB to detect staining and counterstained with Vectors Hemotoxylin. PBS washes (3X2 mins) were done in between all steps except in between the block and the primary

IHC-FFPE sections
I. Deparaffinization:
A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

II. Quench Endogenous Peroxidase:
To Prepare 200 ml of Quenching Solution:
Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol.
**Use within 4 hours of preparation
A. Place slides in peroxidase quenching solution: 15-30 minutes.
B. Place slides in distilled water: 2 changes for 2 minutes each.

III. Retrieve Epitopes:
A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96C.
B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
D. Slowly add distilled water to further cool for 5 minutes.
E. Rinse slides with distilled water, 2 changes for 2 minutes each.

IV. Immunostaining Procedure:
A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).
B. Flood slide with Wash Solution.
Do not allow tissue sections to dry for the rest of the procedure.

C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.

D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.

E. Wash slides with Wash Solution: 3 changes for 5 minutes each.

F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.

G. Wash slides with Wash Solution: 3 changes for 5 minutes each.

H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes.

Check development with microscope.

I. Wash slides with Wash Solution: 3 changes for 5 minutes each.

J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.

K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.

L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.

M. Rinse slides in distilled water.

N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.

O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.

P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.

Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.

R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.

S. Lay slides on a flat surface to dry prior to viewing under microscope.

NOTES:

-Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.

-Prior to Deparaffinization, heat slides overnight in a 60C oven.

-All steps in which Xylene is used should be performed in a fume hood.
- For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.

- For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.

- 200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts, for small tissue sections less than 200 ul may be used.

- 5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.

- Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1.5 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes)
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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