HIF-1 alpha Antibody (H1alpha67)
NB100-123

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

Reviews: 7  Publications: 137

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Updated 10/18/2019 v.20.1

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### Product Information

<table>
<thead>
<tr>
<th><strong>Unit Size</strong></th>
<th>0.1 ml</th>
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<tbody>
<tr>
<td><strong>Concentration</strong></td>
<td>1.0 mg/ml</td>
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<tr>
<td><strong>Storage</strong></td>
<td>Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.</td>
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<tr>
<td><strong>Clonality</strong></td>
<td>Monoclonal</td>
</tr>
<tr>
<td><strong>Clone</strong></td>
<td>H1alpha67</td>
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<tr>
<td><strong>Preservative</strong></td>
<td>0.05% Sodium Azide</td>
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<tr>
<td><strong>Isotype</strong></td>
<td>IgG2b</td>
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<tr>
<td><strong>Purity</strong></td>
<td>Protein A purified</td>
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<tr>
<td><strong>Buffer</strong></td>
<td>PBS with 1% BSA</td>
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<tr>
<td><strong>Target Molecular Weight</strong></td>
<td>93 kDa</td>
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### Product Description

**Host**

Mouse

**Gene ID**

3091

**Gene Symbol**

HIF1A

**Species**

Human, Mouse, Rat, Porcine, Avian, Bovine, Canine, Ferret, Primate, Rabbit, Sheep

**Reactivity Notes**

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. Rabbit reactivity reported in scientific literature (PMID: 16738327, 26339038).

**Immunogen**

This HIF-1 alpha Antibody (H1alpha67) was developed against 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, Immunoblotting, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockout Validated

**Recommended Dilutions**


**Application Notes**

ChIP, Gel Super Shift Assays and ELISA usages were reported in scientific literature. By WB, this antibody recognizes bands at 120kDa representing HIF-1 alpha in induced tissues and cells. Multiple bands may be seen at 120kDa representing post-translational modifications. Nuclear extracts are recommended for WB. Knock Out Validation was reported in scientific literature (PMID: 27991597). Use in Immunoblotting reported in multiple pieces of scientific literature.
Western Blot: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Analysis using the HRP conjugate of NB100-123. Detection of HIF-1 alpha in cobalt chloride treated/untreated COS-7 nuclear extracts using NB100-123.

Western Blot: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Analysis using the HRP conjugate of NB100-123. On day 1, MEF cells (+/+,-/-), were grown on 15cm dish (2x10 to the 6th cells). On day 2, cells were exposed to hypoxia for 4hrs. Cells were washed with ice cold PBS twice and whole cell protein was extracted with RIPA buffer fortified with protease. Upon quantification, 100ug of protein was fractionated on 7% polyacrylamide gel. Gel was transferred overnight onto nitrocellulose membrane. The membrane was probed with HIF-1 alpha monoclonal antibody at a 1:500 dilution (NB100-123). The secondary antibody was conjugated with HRP and was used at a 1:2500 dilution. Photo courtesy of Dr. Gregg Semenza, Johns Hopkins University.

Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Analysis of HIF-1 alpha in human lung tissue. Image courtesy of product review by Aneta Gandjeva.

Western Blot: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Immunoprecipitation of HIF-1alpha using NB100-123. Heavy and light chains are also detected. Image using the HRP form of this antibody.
Immunocytochemistry/Immunofluorescence: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Staining in pig endothelial cells under hypoxia condition using NB100-123. Image from verified customer review.

Immunohistochemistry: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Staining of biotin conjugated HIF-1 alpha (NB100-123B) in human kidney.

Immunohistochemistry-Paraffin: HIF-1 alpha Antibody (H1alpha67) [NB100-123] - Staining on pig tissue (brown) using NB100-123. Image from verified customer review.
<table>
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<tr>
<td>Lee, SM;Lee, KW;Kim, MA;Song, YS;Goo, JM;Park, CM; Serial Texture Analyses on ADC Maps for Evaluation of Antiangiogenic Therapy in Rat Breast Cancer Anticancer Res. Apr 1 2019 12:00AM [PMID: 30952728] (IHC, Rat)</td>
</tr>
<tr>
<td>Timpano S, Guild BD, Specker EJ et al. Physioxic human cell culture improves viability, metabolism, and mitochondrial morphology while reducing DNA damage. FASEB J. Jan 16 2019 12:00AM [PMID: 30649960] (WB, Mouse)</td>
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More publications at [http://www.novusbio.com/NB100-123](http://www.novusbio.com/NB100-123)
**Procedures**

**Western Blot protocol specific for HIF-1 alpha Antibody (NB100-123)**

**Protocol specific for NB100-123 Monoclonal Anti-HIF-1 alpha**

**Western Blot Protocol**

1. Resolve aliquots (25-30 µg) of induced nuclear protein extracts on a 4-20% Tris-HCl gel.
2. Transfer proteins to nitrocellulose membrane in 20 mM Tris-HCL (pH 8.0)/150 mM glycine/20% (vol/vol) methanol.
3. Block membrane for 1 hour with 1X western wash buffer containing 5% non-fat dry milk (NFDM).
4. Incubate membrane overnight at 4C in NB 100-123 diluted 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 membrane with 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.
10. Drain membrane and place on new saran wrap.
11. Wrap up membrane and expose to film.
12. Develop accordingly.

10X Western wash: 24.2 g 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)
To strip membrane: Incubate membrane in stripping buffer for 30 minutes at 56C. Wash membrane for 15 minutes with several change 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.


**Immunohistochemistry Protocol (NB100-123)**

Please see:


**Immunocytochemistry/Immunofluorescence Protocol for HIF-1 alpha Antibody (NB100-123)**

1. Fix cells in 3% Paraformaldehyde in PBS for 15 minutes at room temperature, gently rocking.
2. Rinse cells 3 times for 5 minutes in PBS.
3. Block and permeabilize cells in 2% non-fat dry milk (NFDM) dissolved in PBS with 0.1% TX-100 overnight at 4C (covered to prevent evaporation).
4. Rinse cells 3 times for 5 minutes in PBS.
5. Dilute NB100-123 1:500 in dilution buffer [2% BSA in PBS with 0.01% TX-100].
6. Place cover slip upside down on a 50 ul drop of diluted antibody on parafilm, in humidity box.
7. Incubate for 1 hour at 37C.
8. Flip slips right side up in wells and rinse 3 times for 5 minutes each, in PBS.
9. In an amber microfuge tube, dilute secondary antibody (Cy3 anti-ms IgG) 1:500 in dilution buffer [2% BSA in PBS with 0.01% TX-100].
10. Place 800 ul of diluted secondary antibody in each well and make sure the fluid film covers over the cells on the slip. Alternatively, secondary antibody can be applied in the same manner as the primary (slip upside-down on drop of secondary that has been placed on a sheet of parafilm that is inside of a humidity box).
11. Incubate for 1 hour at 37C, in the dark.
12. Rinse cells at room temperature 4 times for 15 minutes each, in PBS, gently rocking.
14. Refrigerate flat and covered.
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.

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