Product Datasheet

HIF-2 alpha/EPAS1 Antibody (ep190b)
NB100-132

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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# NB100-132
HIF-2 alpha/EPAS1 Antibody (ep190b)

## 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>
</tr>
<tr>
<td>Concentration</td>
<td>1.0 mg/ml</td>
</tr>
<tr>
<td>Storage</td>
<td>Aliquot and store at -20°C or -80°C. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td>Clonality</td>
<td>Monoclonal</td>
</tr>
<tr>
<td>Clone</td>
<td>ep190b</td>
</tr>
<tr>
<td>Preservative</td>
<td>0.05% Sodium Azide</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG1</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>118 kDa</td>
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## Product Description

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host</td>
<td>Mouse</td>
</tr>
<tr>
<td>Gene ID</td>
<td>2034</td>
</tr>
<tr>
<td>Gene Symbol</td>
<td>EPAS1</td>
</tr>
<tr>
<td>Species</td>
<td>Human, Mouse, Rat, Hamster</td>
</tr>
<tr>
<td>Reactivity Notes</td>
<td>Ability to use in mouse is mixed with some positive and some negative results.</td>
</tr>
<tr>
<td>Specificity/Sensitivity</td>
<td>This is specific for HIF-2 alpha and does not cross-react with HIF-1 alpha.</td>
</tr>
<tr>
<td>Immunogen</td>
<td>Human HIF-2 alpha, corresponding to amino acids 535-631. [UniProt# Q99814]</td>
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## Product Application Details

### Applications

Western Blot, Simple Western, Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Gel Supershift Assay, Immunofluorescence

### Recommended Dilutions

Western Blot 1-2 μg/ml, Simple Western 1:100, Chromatin Immunoprecipitation, Flow Cytometry 1:400, ELISA 1:100-1:2000, Immunohistochemistry 1:150-1:300, Immunocytochemistry/Immunofluorescence, Immunoprecipitation, Immunohistochemistry-Paraffin 1:150-1:300, Immunofluorescence, Gel Supershift Assay

### Application Notes

This HIF-2 alpha (ep190b) antibody is useful for ELISA, Flow Cytometry, Immunohistochemistry on paraffin-embedded sections and Western Blot. In WB, it recognizes a band at approx. 118 kDa representing HIF-2 alpha. Use in Gel supper shift assay reported in scientific literature (PMID: 17404621)

In Simple Western only 10 - 15 μL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.
Images

Simple Western: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Lane view shows a specific band for HIF-2 alpha in 0.5 mg/ml of Hypoxic HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

Western Blot: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - HRP conjugated HIF-2 alpha detected in hypoxic human lysate. Lane 1: normoxic A549 lysate control, lane 2: hypoxic A549 lysate.

Immunofluorescence: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Analysis of HIF-2 alpha in human endometrium using anti-HIF-2 alpha antibody. Donkey anti-mouse Alexa Fluor 488 secondary antibody was used. Image from verified customer review.

Flow Cytometry: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - HIF-2 alpha antibody was tested at 1:400 in HepG2 cells using an Alexa Fluor 488 secondary (shown in purple). M1 is defined by unstained cells.
Western Blot: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - [HRP] [NB100-132H] - Analysis of HIF-2 alpha stabilization over time in HOS cells following exposure to hypoxia. [PMID: 23785417] Image using the HRP form of this antibody.

Immunohistochemistry-Paraffin: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Analysis of HIF-2 in human cardiac myocytes.

Western Blot: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Analysis of HepG2 without Cobalt (II) Chloride (1), HepG2 with Cobalt (II) Chloride (2), HepG2 normoxic (3), HepG2 hypoxic (4), HepG2 without Cobalt (II) Chloride (5), HepG2 with Cobalt (II) Chloride (6), HepG2 normoxic (7), and HepG2 hypoxic (8) using HIF-2 alpha antibody (NB100-132) at 1 - 2 ug/ml.

Western Blot: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - C57B6 5 week old Mouse Kidney Tissue 60ug. Image from verified customer review.
Western Blot: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Analysis of HIF-2 alpha stabilization over time in 791T cells following exposure to hypoxia. [PMID: 23785417] Image using the HRP form of this antibody.

Immunohistochemistry-Paraffin: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Analysis of HIF-2 alpha in paraffin-embedded mouse kidney tissue using anti-HIF-2 alpha antibody. Image from verified customer review.
<table>
<thead>
<tr>
<th>Publication</th>
<th>Authors</th>
<th>Title</th>
<th>Date</th>
<th>PMID</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developmental vascular pruning in neonatal mouse retinas is programmed in the astrocytic oxygen sensing mechanism</td>
<td>Duan LJ, Fong GH.</td>
<td>Development                                                Mar 25 2019 12:00AM [PMID: 30910827] (WB, Mouse)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arrest-defective-1 protein, an acetyltransferase, does not alter stability of hypoxia-inducible factor (HIF)-1alpha and is not induced by hypoxia or HIF.</td>
<td>Bilton R, Mazure N, Trottier E et al.</td>
<td>J Biol Chem.                                             Sep 02 [PMID: 15994306] (WB, Human)</td>
<td></td>
<td></td>
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</tbody>
</table>

Procedures

Immunohistochemistry Protocol for HIF-2 alpha Antibody (NB100-132)

Procedure Guide for NB100-132

Monoclonal Anti-HIF-2 alpha Western Blot Procedure
1. Resolve nuclear cell extracts (50-100 ug/lane) on a 6% SDS-polyacrylamide gel, under reducing conditions.
2. Transfer to a nitrocellulose membrane, overnight, or to a *PVDF membrane [*in 20 mM Tris/100 mM glycine/10% (v/v) methanol/0.05% SDS].
3. Block the membrane in TBS containing 5% non-fat dry milk and 0.1% Tween-20.
4. Rinse the membrane in TBST, twice.
5. Incubate the membrane in anti-HIF-2 alpha (NB100-132), diluted 1:500 in TBS+1% BSA, overnight at 4C.
6. Wash membrane with TBST for 35 minutes at RT (1 X 15 minutes, 2 X 10 minutes).
7. Incubate the membrane with diluted HRP conjugated goat anti-mouse antibody.
8. Wash membrane with TBST for 35 minutes at RT (1 X 15 minutes, 2 X 10 minutes).
9. Use Amersham ECL Kit, as directed, to detect image.

Immunohistochemistry Procedure for Paraffin Sections
1. Prior to performing the IPOX experiment, dewax the paraffin sections by baking them at 60C for 30 minutes and then putting them through citroclear.
2. Hydrate the 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. Block endogenous peroxidase with 0.5% hydrogen peroxide in water, for 30 minutes.
4. Antigen unmasking is performed by incubating at 60C for 16 hours, in 50mmol/L Tris and 0.2 mmol/L EDTA (pH 9.0), using a covered water bath.
5. Rinse slides with PBS and then incubate with PBS containing 0.2% Triton X-100 for 10 minutes.
6. Rinse slides with PBS.
7. Incubate sections with 1:1,000-1:3,000 dilution of anti-HIF-2 alpha (NB 100-132) for 90 minutes at RT.
8. Incubate sections in secondary HRP-conjugated goat anti-mouse serum for 30 minutes at RT.
9. Incubate sections in tertiary HRP-conjugated rabbit anti-goat serum for 30 minutes at RT.
10. Develop the peroxidase reaction using diaminobenzidine.
11. Wash slide and mount in aqueous mountant.

Substitution of the primary antibody with PBS can be used as a negative control.
1. Sub-confluent cells are grown on chamber slides and incubated for 16 hours either in air or under 0.1% hypoxia.
2. Wash cells in ice-cold PBS.
3. Fix cells in formaldehyde (3.7% in PBS) for 10 minutes at room temperature (RT).
4. Wash cells twice, in PBS, and permeabilize by incubating in 0.2% Triton X-100 in PBS for 10 minutes at RT.
5. Incubate the slides with 1:1,000-1:3,000 dilution of anti-HIF-2 alpha (NB 100-132) for 1 hour at RT.
6. Wash in PBS for 5 minutes.
7. Incubate with HRP-conjugated goat anti-mouse for 30 minutes at RT.
9. Counterstain with hematoxylin.
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:
   A. Place slides in peroxidase quenching solution: 15-30 minutes.
   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
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 90-96 degrees C.
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 60 degrees C 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 1/2 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.

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