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><strong>Unit Size</strong></th>
<th>0.1 ml</th>
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<tr>
<td><strong>Concentration</strong></td>
<td>1.0 mg/ml</td>
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<td><strong>Storage</strong></td>
<td>Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.</td>
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<tr>
<td><strong>Clonality</strong></td>
<td>Monoclonal</td>
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<tr>
<td><strong>Clone</strong></td>
<td>ep190b</td>
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<tr>
<td><strong>Preservative</strong></td>
<td>0.05% Sodium Azide</td>
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<td><strong>Isotype</strong></td>
<td>IgG1</td>
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<tr>
<td><strong>Purity</strong></td>
<td>Protein G purified</td>
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<td><strong>Buffer</strong></td>
<td>PBS, 1% BSA</td>
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<td><strong>Target Molecular Weight</strong></td>
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### Product Description

| **Host** | Mouse |
| **Gene ID** | 2034 |
| **Gene Symbol** | EPAS1 |
| **Species** | Human, Mouse, Rat, Bovine, Hamster |
| **Reactivity Notes** | Ability to use HIF-2 alpha/EPAS1 Antibody (ep190b) in mouse is mixed with some positive and some negative results. Use in Bovine reported in scientific literature (PMID:32054096). |
| **Specificity/Sensitivity** | This HIF-2 alpha/EPAS1 Antibody (ep190b) is specific for HIF-2 alpha/EPAS1, and does not cross-react with HIF-1 alpha. |
| **Immunogen** | The immunogen recognized by this HIF-2 alpha/EPAS1 Antibody (ep190b) maps to a region between amino acids 535-631. [UniProt# Q99814] |

### Product Application Details

| **Applications** | Western Blot, Simple Western, ELISA, Flow Cytometry, Gel Super Shift Assays, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, In vivo assay, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Gel Supershift Assay, Knockdown Validated |
| **Recommended Dilutions** | Western Blot 1 - 2 ug/mL, Simple Western 1:100, Flow Cytometry 1:400, ELISA 1:100-1:2000, Immunohistochemistry 1:150 - 1:300, Immunocytochemistry/Immunofluorescence, Immunoprecipitation, Immunohistochemistry-Paraffin 1:150 - 1:300, Immunohistochemistry-Frozen reported in scientific literature (PMID 24973414), Gel Super Shift Assays reported in scientific literature (PMID 17404621), In vivo assay reported in scientific literature (PMID 23857308), Gel Supershift Assay, Chromatin Immunoprecipitation (ChIP), Knockdown Validated reported in scientific literature (PMID 32054096) |
| **Application Notes** | In WB, it recognizes a band at approx. 118 kDa representing HIF-2 alpha.  
In Simple Western only 10 - 15 uL 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. |
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] - Analysis of HIF-2 alpha stabilization over time in 791T cells following exposure to hypoxia. Image using the HRP form of this antibody (NB100-132H). Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/23785417/) licensed under a CC-BY license.

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

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] - Mouse aortic endothelial cells treated (1%) or not treated (20.9%) in hypoxia for 3 hrs. Cells where also transfected with a specific siRNA against (siHIF-2) or a control siRNA (-). Western blot image submitted by a verified customer review.

Immunohistochemistry-Paraffin: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Renal tubule specific models of Vhl deletion. Histological images of representative renal sections from 12 month old control, Pax8-CreERT2/Vhl-delta/delta and Slc22a6-CreERT2/Vhl-delta/delta mice (stains and antibodies as indicated, arrowheads indicate abnormal vascularization). Scale bars, 100 um. Image collected and cropped by CiteAb from the following publication (http://dx.plos.org/10.1371/journal.pone.0148055), licensed under a CC-BY license.

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

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. Image using the HRP form of this antibody (NB100-132H). Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/23785417/) licensed under a CC-BY license.
Western Blot: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Western blot analysis and quantification of HIF-2 alpha expression in the cortex 4.5 hours after lipopolysaccharide (LPS) administration for all LPS groups and the control group (SHAM, white bar). With the exception of the vagotomy group (VGX LPS, gray bar), no significant differences to the SHAM group were found in the LPS-treated and sham-operated (SHAM+LPS, light gray bar) or vagus nerve-stimulated groups (VGX LPS+STIM, black bar). The significant increase in the VGX LPS (gray bar) group is an indicator of a hypoxic condition; * P<0.05 compared to SHAM; n=6 rats each. Data are given as the mean+/−SEM. Image collected and cropped by CiteAb from the following publication (http://jneuroinflammation.biomedcentral.com/articles/10.1186/1742-2094-9-183), licensed under a CC-BY license.

Western Blot: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - 4-OOHCPA exposure induced HIF-2 alpha/EPAS1. A: Western blot analysis of HIF-2 alpha/EPAS1 protein (118KD) and actin (43KD) in limbs at 10 min, 1, 3, 6, and 24 h after treatment with 4-OOHCPA at 0.3 ug/mL (L) 1.0 ug/mL (M) or 3.0 ug/mL (H). P represents the positive control. Image collected and cropped by CiteAb from the following publication (http://dx.plos.org/10.1371/journal.pone.0051937), licensed under a CC-BY license.

Immunohistochemistry: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Analysis of HIF-2 alpha in human endometrium using. Donkey anti-mouse Alexa Fluor 488 secondary antibody was used. IHC image submitted by a verified customer review.

Knockdown Validated: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Functional role of HIF2A in the transcriptional regulation of amphiregulin (AREG) in human cardiac myocytes. Immunoblot for HIF1A or HIF2A from shRNA-transfected normoxic or hypoxic HCM. Beta-Actin (ACTb) served as a loading control. Image collected and cropped by CiteAb from the following publication (http://pubmed.ncbi.nlm.nih.gov/29483579/) licensed under a CC-BY license.
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Title</th>
<th>Journal</th>
<th>Date</th>
<th>PubMed ID</th>
<th>Techniques</th>
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<tr>
<td>Zhou X, Zheng Y, Sun W et al.</td>
<td>D-mannose alleviates osteoarthritis progression by inhibiting chondrocyte ferroptosis in a HIF-2α-dependent manner</td>
<td>Cell Proliferation</td>
<td>2021-11-01</td>
<td>34561933</td>
<td>ICC/IF, WB, IHC</td>
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<td>Akahori D, Inui N, Inoue Y et al.</td>
<td>Effect of Hypoxia on Pulmonary Endothelial Cells from Bleomycin-Induced Pulmonary Fibrosis Model Mice</td>
<td>International journal of molecular sciences</td>
<td>2022-08-12</td>
<td>36012260</td>
<td>ICC/IF, Mouse</td>
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<td>Hulley PA, Papadimitriou-Olivgeri I, Knowles HJ</td>
<td>Osteoblast-osteoclast co-culture amplifies inhibitory effects of FG-4592 on osteoclast formation and reduces bone resorption activity</td>
<td>JBMR Plus</td>
<td>2020-07-16</td>
<td>32666021</td>
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<td>Niklasson, C U, Fredlund, E Et al.</td>
<td>Hypoxia inducible factor-2 alpha importance for migration, proliferation, and self-renewal of trunk neural crest cells.</td>
<td>Dev Dyn</td>
<td>2021-02-01</td>
<td>32940375</td>
<td>WB, Mouse</td>
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<td>Kuwano A, Kurokawa M, Kohjima M et al.</td>
<td>Microcirculatory disturbance in acute liver injury</td>
<td>Experimental and therapeutic medicine</td>
<td>2021-06-01</td>
<td>33884034</td>
<td>IF/IHC, Human</td>
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<tr>
<td>Rodriguez L, Duchez P, Touya N et al.</td>
<td>alpha-Tocopherol Attenuates Oxidative Phosphorylation of CD34+ Cells, Enhances Their G0 Phase Fraction and Promotes Hematopoietic Stem and Primitive Progenitor Cell Maintenance</td>
<td>Biomolecules</td>
<td>2021-04-10</td>
<td>33920203</td>
<td>WB, Mouse</td>
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<tr>
<td>Pavlakis D, Kampantais S, Gkagkalidis K et al.</td>
<td>Hypoxia-Inducible Factor 2α Expression Is Positively Correlated With Gleason Score in Prostate Cancer</td>
<td>Technology in cancer research &amp; treatment</td>
<td>2021-03-23</td>
<td>33752529</td>
<td>IHC-P, Human</td>
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<tr>
<td>Wang X, Schneider A</td>
<td>HIF-2alpha-mediated activation of the epidermal growth factor receptor potentiates head and neck cancer cell migration in response to hypoxia.</td>
<td>Carcinogenesis</td>
<td>2010-01-07</td>
<td>20395290</td>
<td>IF/IHC, Human</td>
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**Procedures**

**Immunohistochemistry Protocol for HIF-2 alpha Antibody (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 (NB 100-132), diluted 1:500 in TBS+1% BSA, overnight at 4°C.
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 60°C 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 60°C 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
- 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 1-1.5 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](http://www.novusbio.com/guarantee)

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<th>Description</th>
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<td>NB820-59231</td>
<td>Human Kidney Whole Tissue Lysate (Adult Whole Normal)</td>
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<td>HAF007</td>
<td>Goat anti-Mouse IgG Secondary Antibody [HRP]</td>
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<td>NB720-B</td>
<td>Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]</td>
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<tr>
<td>NBP1-97005-0.5mg</td>
<td>Mouse IgG1 Isotype Control (MG1)</td>
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**Bio-Techne Canada**

21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

**Bio-Techne Ltd**

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.EMEA@bio-techne.com

**General Contact Information**

[www.novusbio.com](http://www.novusbio.com)
Technical Support: nb-technical@bio-techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com