

# **Product Datasheet**

## **HIF-2 alpha/EPAS1 Antibody**

### **NB100-132SS**

Unit Size: 0.025 ml

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

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Updated 6/15/2014 v.20.1

**NB100-132SS**

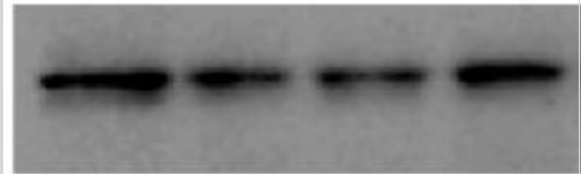
HIF-2 alpha/EPAS1 Antibody (ep190b)

<b>Product Information</b>	
<b>Unit Size</b>	0.025 ml
<b>Concentration</b>	1.257 mg/ml
<b>Storage</b>	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
<b>Clonality</b>	Monoclonal
<b>Clone</b>	ep190b
<b>Preservative</b>	0.1% Sodium Azide
<b>Isotype</b>	IgG1
<b>Purity</b>	Protein G purified
<b>Buffer</b>	PBS
<b>Target Molecular Weight</b>	118 kDa
<b>Product Description</b>	
<b>Host</b>	Mouse
<b>Gene ID</b>	2034
<b>Gene Symbol</b>	EPAS1
<b>Species</b>	Human, Mouse, Rat, Hamster
<b>Species Reactivity</b>	Human, mouse, rat and hamster. Ability to use in mouse is mixed with some positive and some negative results.
<b>Specificity/Sensitivity</b>	This is specific for HIF-2 alpha and does not cross-react with HIF-1 alpha.
<b>Immunogen</b>	Human HIF-2 alpha, corresponding to amino acids 535-631. [UniProt# Q99814]
<b>Product Application Details</b>	
<b>Applications</b>	Western Blot, Simple Western, Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
<b>Recommended Dilutions</b>	ELISA 1:100-1:2000, Immunohistochemistry 1:150-1:300, Immunohistochemistry-Paraffin 1:150-1:300, Chromatin Immunoprecipitation, Immunocytochemistry/Immunofluorescence, Immunoprecipitation, Flow Cytometry 1:400, Western Blot 1-2 ug/ml, Simple Western 1:100
<b>Application Notes</b>	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 ~118 kDa representing HIF-2 alpha. In Simple Western only 10-15 uL of the recommended dilution is used per data point.

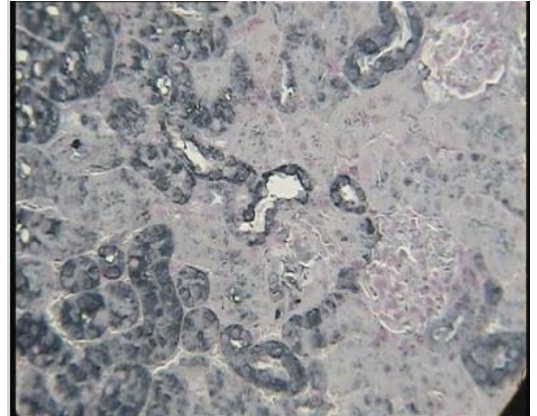


## Images

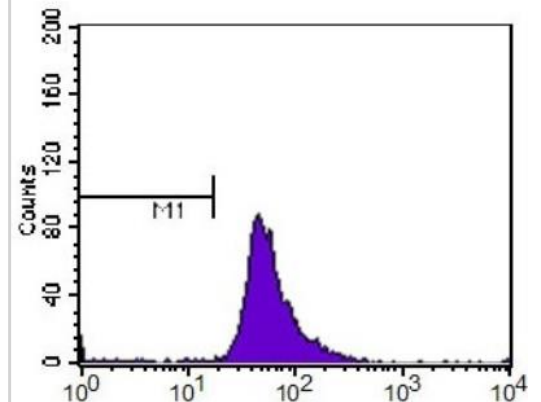
Western Blot: HIF-2 alpha Antibody (ep190b) [NB100-132] - C57B6 5 week old Mouse Kidney Tissue 60ug. Image from verified customer review.



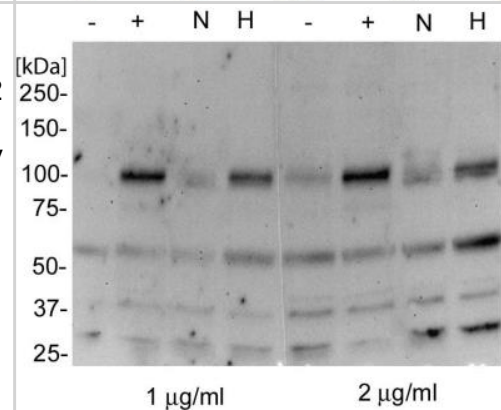
Immunohistochemistry-Paraffin: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - analysis of HIF-2 alpha in paraffin-embedded mouse kidney tissue using anti-HIF-2 alpha antibody. Image from verified customer review.



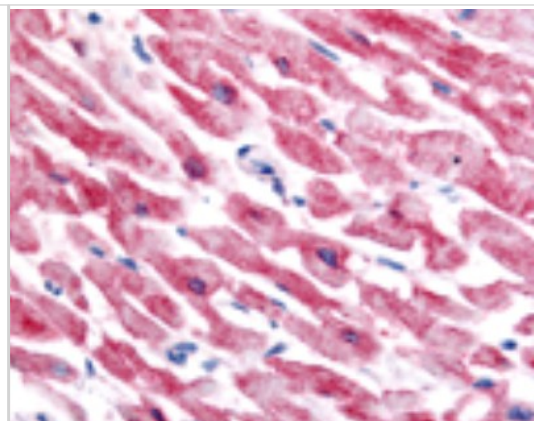
Flow Cytometry: HIF-2 alpha Antibody (ep190b) [NB100-132] - HIF-2 alpha antibody was tested at 1:400 in HepG2 cells using an Alexa Fluor 488 secondary (shown in purple). M1 is defined by unstained cells.



Western Blot: HIF-2 alpha Antibody (ep190b) [NB100-132] - Western blot 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.



Immunohistochemistry: HIF-2 alpha Antibody (ep190b) [NB100-132] - Staining of heart, cardiac myocytes.



Simple Western: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Simple Western 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.



## Publications

Xu M, Nagati JS, Xie J et al. An acetate switch regulates stress erythropoiesis. *Nat. Med.* 2014 Aug 10 [PMID: 25108527] (WB, ChIP, IP, Human)

Evans CE, Wadoodi A, Humphries J et al. Local accumulation of hypoxia-inducible factor 2 alpha during venous thrombus resolution. *Thromb. Res.* 2014 Jul 23 [PMID: 25087887] (IHC-P, Mouse)

Details:  
HIF2a/HIF-2 alpha antibody used for IHC-P in venous thrombus induced in the inferior vena cava /IVC of male BALB/C mice treated or not with HIF agonist L-mimosine (Fig 1, 2). Antigen retrieval - pressure cooking sections in citrate buffer (0.3% sodium citrate, pH 6).

Peng YJ, Yuan G, Khan S et al. Regulation of Hypoxia Inducible Factor-alpha Isoforms and Redox State By Carotid Body Neural Activity in Rats. *J. Physiol. (Lond.)*. 2014 Jun 27 [PMID: 24973414] (IHC-Fr, WB, Rat)

Carrera S, Senra J, Acosta MI et al. The Role of the HIF-1alpha Transcription Factor in Increased Cell Division at Physiological Oxygen Tensions. *PLoS ONE*. 2014 May 19 [PMID: 24835245] (WB, Human)

Toyoda H, Hirayama J, Sugimoto Y et al. Polycythemia and Paraganglioma With a Novel Somatic HIF2A Mutation in a Male. *Pediatrics*. 2014 May 12 [PMID: 24819565] (IHC-P, Human)

Gregg JL, Turner RM, Chang G et al. NADPH oxidase NOX4 supports renal tumorigenesis by promoting the expression and nuclear accumulation of HIF2alpha. *Cancer Res.* 2014 Apr 22 [PMID: 24755467] (ICC/IF, IHC-P, WB, Human)

Raspaglio G, Petrillo M, Martinelli E et al. Sox9 and Hif-2a regulate TUBB3 gene expression and affect ovarian cancer aggressiveness. *Gene* 3/21/2014 [PMID: 24661907] (ChIP, WB, Human)

Duan LJ, Takeda K, Fong GH. Hypoxia inducible factor-2 alpha regulates the development of retinal astrocytic network by maintaining adequate supply of astrocyte progenitors. *PLoS ONE* 2014 Jan 29 [PMID: 24475033] (WB, Mouse)

Duan LJ, Takeda K, Fong GH. Hematological, Hepatic, and Retinal Phenotypes in Mice Deficient for Prolyl Hydroxylase Domain (PHD) Proteins in the Liver. *Am. J. Pathol.* 2014 Feb 19 [PMID: 24508125] (WB, Mouse)

Anderson ER, Taylor M, Xue X et al. Intestinal HIF2alpha promotes tissue-iron accumulation in disorders of iron overload with anemia. *Proc Natl Acad Sci U S A.* 2013 Dec 10 [PMID: 24282296] (WB, Mouse)

Thompson AA, Elks PM, Marriott HM et al. Hypoxia-inducible factor 2alpha regulates key neutrophil functions in humans, mice and zebrafish. *Blood.* 2013 Nov 6 [PMID: 24196071] (WB, IHC, Human, Mouse)

Zhang T, Niu X, Liao L et al. The Contributions of HIF-Target Genes to Tumor Growth in RCC. *PLoS One.* 2013 Nov 18 [PMID: 24260413] (WB, Human)

More publications at <http://www.novusbio.com/NB100-132>



## Procedures

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

Procedure Guide for NB 100-132

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 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.
8. Detect binding using 3Prime-diaminobenzidine.
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).







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### **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](http://www.novusbio.com/guarantee).**

