Product Datasheet

HIF-2 alpha/EPAS1 Antibody
NB100-122

Unit Size: 0.1 ml

Store at 4C. Do not freeze.

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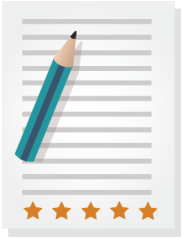


NB100-122

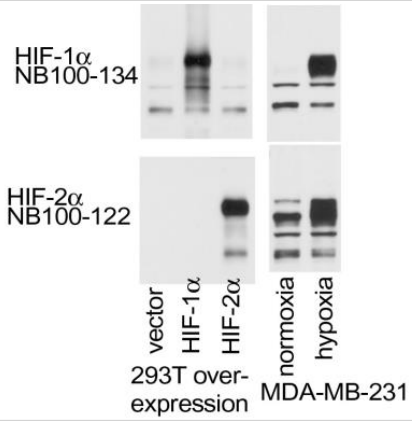
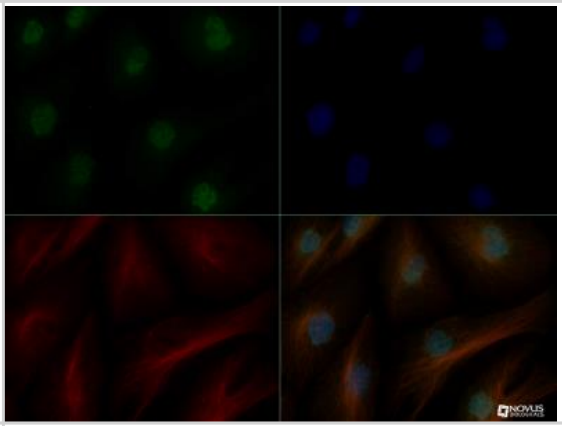
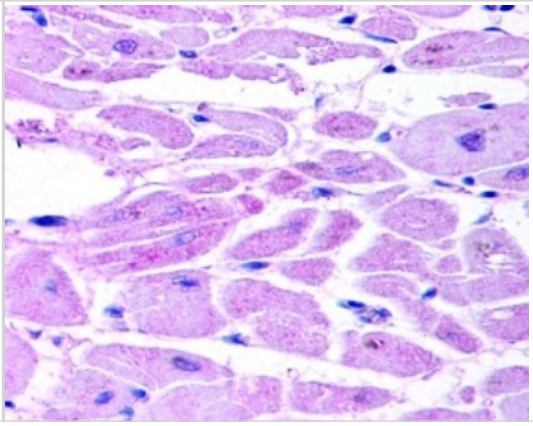
HIF-2 alpha/EPAS1 Antibody

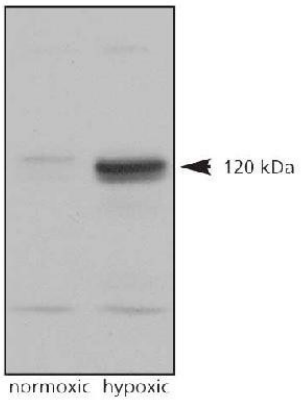
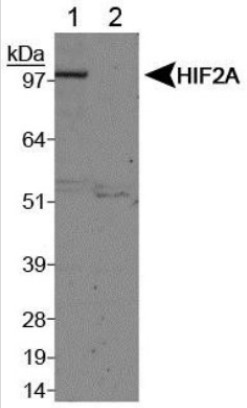
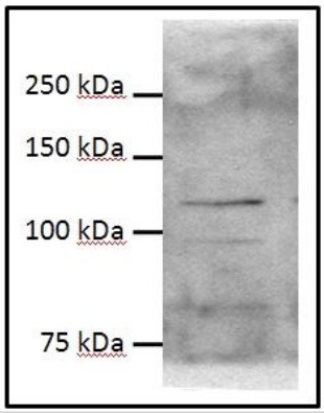
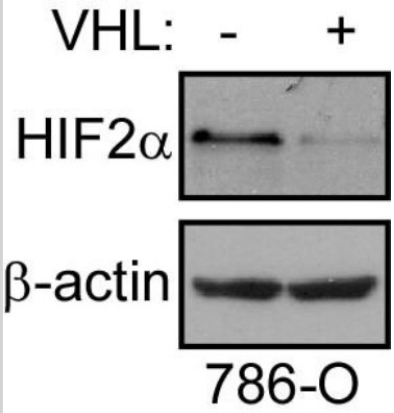
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	118 kDa

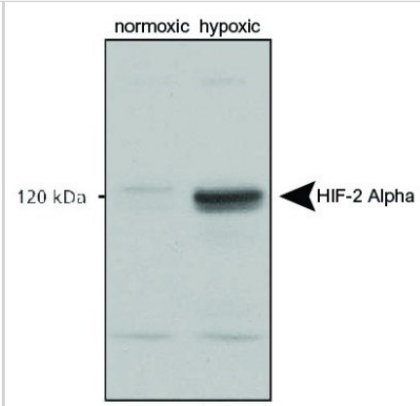
Product Description	
Host	Rabbit
Gene ID	2034
Gene Symbol	EPAS1
Species	Human, Mouse, Rat, Fish, Hamster, Primate, Sheep
Reactivity Notes	Reactivity with sheep reported in the scientific literature (PMID: 17307811). Hamster reactivity reported in scientific literature (PMID: 24997364 and 24997360)
Specificity/Sensitivity	Specific for HIF-2 alpha/EPAS. Does not cross-react with HIF-1 alpha.
Immunogen	A peptide derived from the C-terminus of mouse/human HIF-2 alpha protein.

Product Application Details	
Applications	Western Blot, Simple Western, Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Gel Super Shift Assays, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Western Blot 1.0 ug/ml - 2.0 ug/ml, Simple Western 1:50, Chromatin Immunoprecipitation 1:10 - 1:500, Flow Cytometry, ELISA 1:100 - 1:2000, Immunohistochemistry 1:100, Immunocytochemistry/Immunofluorescence 1:100, Immunoprecipitation 5ug/1mg lysate, Immunohistochemistry-Paraffin 1:100, Immunohistochemistry-Frozen, Gel Super Shift Assays
Application Notes	This HIF-2 alpha antibody is useful for Western blot, Immunoprecipitation, Chromatin Immunoprecipitation, ELISA, Gel Shift Super Assay (PMID: 15184875), Immunocytochemistry/Immunofluorescence and Immunohistochemistry on paraffin-embedded sections. In WB, this antibody recognizes a band at 118kDa 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.

Images

Western Blot: HIF-2 alpha/EPAS1 Antibody [NB100-122] - Analysis of HIF-2 alpha in human Retinal and Choroidal primary endothelia lysates using anti-HIF-2 alpha antibody. Image from verified customer review.	
Western Blot: HIF-2 alpha/EPAS1 Antibody [NB100-122] - Analysis of HIF-2 alpha in MDA-MB-231 cell lysate (overexpression and endogenous samples) using anti-HIF-2 alpha antibody. The data showed that HIF-2 alpha antibody did not react to HIF-1 alpha overexpression. Image from verified customer review.	
Immunocytochemistry/Immunofluorescence: HIF-2 alpha/EPAS1 Antibody [NB100-122] - HIF-2 alpha antibody was tested in RCC4 cells at a 1:200 dilution against Dylight 488 (Green). Alpha tubulin and nuclei were counterstained against Dylight 568 (Red) and DAPI (Blue), respectively.	
Immunohistochemistry-Paraffin: HIF-2 alpha/EPAS1 Antibody [NB100-122] - Hif-2 alpha immunoreactivity in human cardiac myocytes stained with NB100-122.	

Western Blot: HIF-2 alpha/EPAS1 Antibody [NB100-122] - Analysis on normoxic and hypoxic nuclear rat cell lysates.	
Western Blot: HIF-2 alpha/EPAS1 Antibody [NB100-122] - Analysis of HIF-2 alpha on Lane 1, Cobalt chloride treated COS7 nuclear extracts and Lane 2, Untreated COS7 nuclear extracts using NB100-122.	
Western Blot: HIF-2 alpha/EPAS1 Antibody [NB100-122] - Western Blot on Human Glioma cells, whole cell lysates from a customer review.	
Western Blot: HIF-2 alpha/EPAS1 Antibody [NB100-122] - Western blot of 786-O cells without or with VHL overexpression. Image from verified customer review.	

Western Blot: HIF-2 alpha/EPAS1 Antibody [NB100-122] - Analysis using the HRP conjugate of NB100-122. Detection of normoxic and hypoxic nuclear rat cell lysates.	
Simple Western: HIF-2 alpha/EPAS1 Antibody [NB100-122] - 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

Kuan II, Liang KH, Wang YP et al. EpEX/EpCAM and Oct4 or Klf4 alone are sufficient to generate induced pluripotent stem cells through STAT3 and HIF2alpha. Sci Rep. 2017 Feb 03 [PMID: 28157205]

Bhanu NV, Sidoli S, Garcia BA. Histone modification profiling reveals differential signatures associated with human embryonic stem cell self-renewal and differentiation. Proteomics. 2016 Feb 01 [PMID: 26631989]

Gardner PJ, Liyanage SE, Cristante E et al. Hypoxia inducible factors are dispensable for myeloid cell migration into the inflamed mouse eye Sci Rep Jan 23 2017 12:00AM [PMID: 28112274] (WB, Mouse)

Yamamura K, Uruno T, Shiraishi A et al. The transcription factor EPAS1 links DOCK8 deficiency to atopic skin inflammation via IL-31 induction Nat Commun Jan 9 2017 12:00AM [PMID: 28067314] (ICC/IF, Mouse)

Hoppe-Seyler K, Bossler F, Lohrey C et al. Induction of dormancy in hypoxic human papillomavirus-positive cancer cells Proc. Natl. Acad. Sci. U.S.A Jan 23 2017 12:00AM [PMID: 28115701] (IB, Human)

Mao XG, Wang C, Liu DY et al. Hypoxia upregulates HIG2 expression and contributes to bevacizumab resistance in glioblastoma Oncotarget Jul 26 2016 12:00AM [PMID: 27329597] (ChIP, Human)

Lu H, Chen I, Shimoda LA et al. Chemotherapy-Induced Ca2+ Release Stimulates Breast Cancer Stem Cell Enrichment. Cell Rep. [PMID: 28228260] (WB, ChIP, Human)

Nakayama T, Otsuka S, Kobayashi T et al. Dormant cancer cells accumulate high protoporphyrin IX levels and are sensitive to 5-aminolevulinic acid-based photodynamic therapy. Sci Rep. Nov 18 2016 12:00AM [PMID: 27857072] (WB, Human)

Thompson JM, Nguyen QH, Singh M et al. Rho-associated kinase 1 inhibition is synthetically lethal with von Hippel-Lindau deficiency in clear cell renal cell carcinoma. Oncogene. Nov 14 2016 12:00AM [PMID: 27841867]

Zhang L, Du J, Justus S et al. Reprogramming metabolism by targeting sirtuin 6 attenuates retinal degeneration. J. Clin. Invest. Dec 1 2016 12:00AM [PMID: 27841758] (WB, Mouse)

Isono T, Chano T, Yoshida T et al. Hydroxyl-HIF2-alpha is potential therapeutic target for renal cell carcinomas. Am J Cancer Res. Nov 8 2016 12:00AM [PMID: 27822416] (Human)

Ke S, Chen S, Dong Z et al. Erythrocytosis in hepatocellular carcinoma portends poor prognosis by respiratory dysfunction secondary to mitochondrial DNA mutations. Hepatology. Oct 24 2016 12:00AM [PMID: 27774607]

More publications at http://www.novusbio.com/NB100-122

Procedures

Protocol specific for HIF-2 alpha Antibody (NB100-122)
Procedure Guide for NB 100-122
Polyclonal anti-HIF-2 alpha Western Blot Procedure
1. Resolve aliquots (at least 25ug) of induced nuclear protein extracts on a SDS/4-15% polyacrylamide gel.
2. Transfer to nitrocellulose membranes in 20 mM Tris-HCl (pH 8.0)/150 mM glycine/20% (vol/vol) methanol.
3. Block membranes for 1.5 hours with 1X western wash buffer containing 5% non-fat dry milk (NFDM) and 1% BSA in TBST.
4. Incubate membranes overnight at 4C in NB 100-122 diluted (2.0ug/ml) in 1X western wash bufer 5% NFDM and 1%BSA in TBST.
5. Wash with TBST 5 x 5 mins each.
6. Incubate membranes with HRP conjugated anti-Rabbit IgG for 1 hour (RT) in 1X western wash buffer 5% NFDM and 1% BSA in TBST.
7. Wash with TBST for 5 x 5 mins each.
8. Drain membrane and place on saran wrap or other imaging container.
9. Prepare detection reagent (ECL). Place membrane in solution for 5 mins at room temperature (RT).
10. Drain membrane and place in imaging package or container.
11. Expose to film or imager. Start with quick exposures and increase time as needed.
12. Develop accordingly
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-2alpha has a very short half-life. Nuclear extracts of hypoxia induced cell lines (293, Hep3B, COS7, HeLa) are useful as a positive control.
Nuclear Extract Preparation Reference: Wang and Semenza. Purification and Characterization of Hypoxia-Inducible Factor 1. Journal of Biological Chemistry. 270(3): 1230-1237, 1995.
1. 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 ½ 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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Products Related to NB100-122

NB800-PC26	COS-7 Nuclear Hypoxic Induced Cell Lysate
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NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NB100-122H	HIF-2 alpha/EPAS1 Antibody [HRP]

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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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