Product Datasheet

HIF-2 alpha/EPAS1 Antibody - BSA Free
NB100-122

Unit Size: 0.1 ml

Store at -20 °C.

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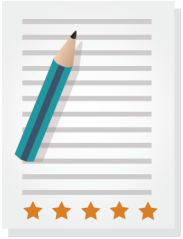
Updated 9/9/2025 v.20.1



NB100-122

HIF-2 alpha/EPAS1 Antibody - BSA Free

Product Information

Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at -20 °C.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	96.5 kDa

Product Description

Description	Novus Biologicals Knockout (KO) Validated Rabbit HIF-2 alpha/EPAS1 Antibody - BSA Free (NB100-122) is a polyclonal antibody validated for use in IHC, WB, ELISA, Flow, Dual RNAscope ISH-IHC, ICC/IF, Simple Western, IP and ChIP. Anti-HIF-2 alpha/EPAS1 Antibody: Cited in 840 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	2034
Gene Symbol	EPAS1
Species	Human, Mouse, Rat, Fish, Hamster, Primate, Rabbit, Reptile, Sheep
Reactivity Notes	Use in Mouse reported in scientific literature (PMID:33758176).
Specificity/Sensitivity	This HIF-2 alpha/EPAS1 Antibody is specific for HIF-2 alpha/EPAS, and does not cross-react with HIF-1 alpha.
Immunogen	This HIF-2 alpha/EPAS1 Antibody was developed against a peptide derived from the C-terminus of mouse/human HIF-2 alpha protein.

Product Application Details

Applications	Western Blot, Simple Western, Immunohistochemistry-Paraffin, ELISA, Flow Cytometry, Gel Super Shift Assays, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, In vitro assay, Immunoprecipitation, SDS-Page, Chromatin Immunoprecipitation (ChIP), Dual RNAscope ISH-IHC, Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 1 - 2 ug/mL, Simple Western 1:50, Flow Cytometry, ELISA 1:100 - 1:2000, Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence 1:100, Immunoprecipitation 5 ug / 1 mg lysate, Immunohistochemistry-Paraffin 1:100, Immunohistochemistry-Frozen, Immunoblotting reported in scientific literature (PMID 28115701), In vitro assay reported in scientific literature (PMID 24998849), Gel Super Shift Assays reported in scientific literature (PMID 15184875), SDS-Page, Chromatin Immunoprecipitation (ChIP) 1:10-1:500, Knockout Validated reported in scientific literature (PMID 26861754), Knockdown Validated reported in scientific literature (PMID 31061092), Dual RNAscope ISH-IHC
Application Notes	In WB, this antibody recognizes a band at 118 kDa representing HIF-2 alpha. See Simple Western Antibody Database for Simple Western validation: tested in hypoxic HeLa lysate (0.5 mg/ml); separated by Size- Wes/Sally Sue/Peggy Sue; antibody dilution of 1:50; apparent MW in kDa on Simple Western was 110kDa; matrix was 12-230 kDa.

Images

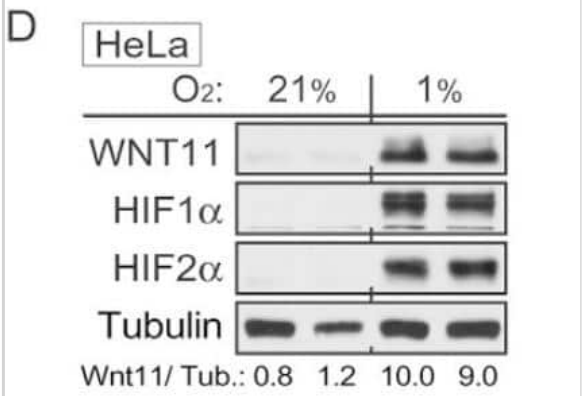
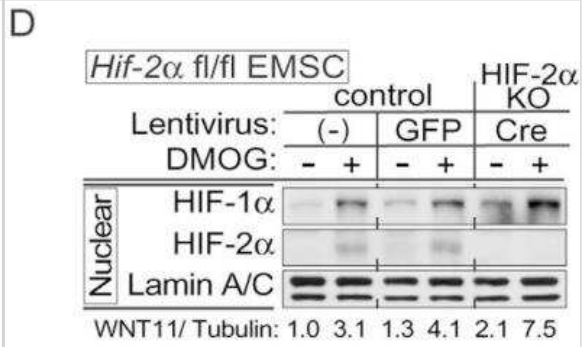
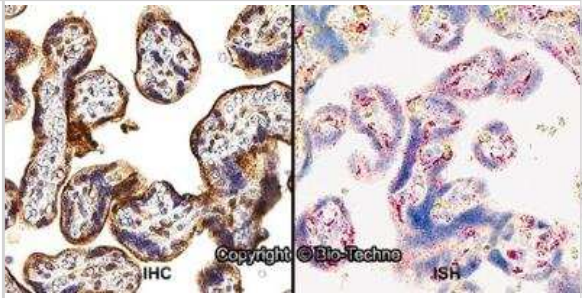
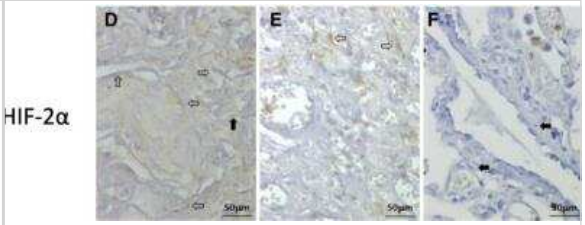
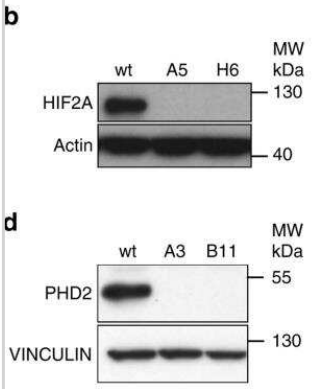
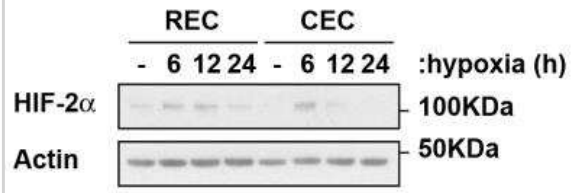
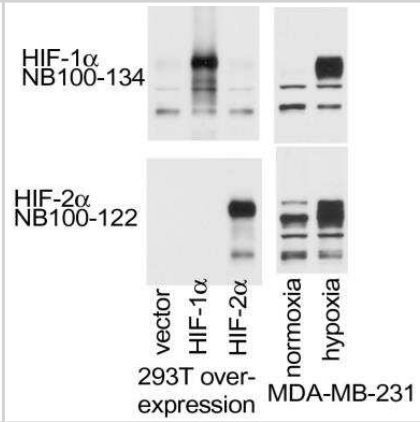
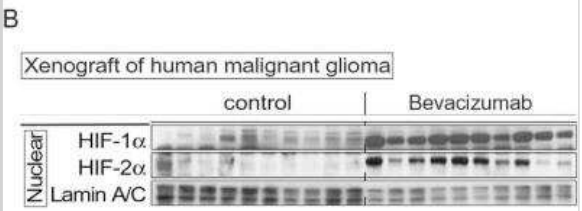
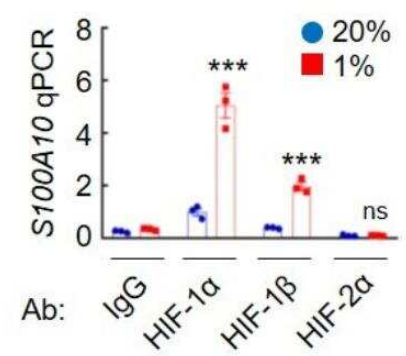
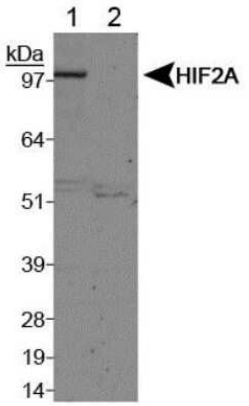
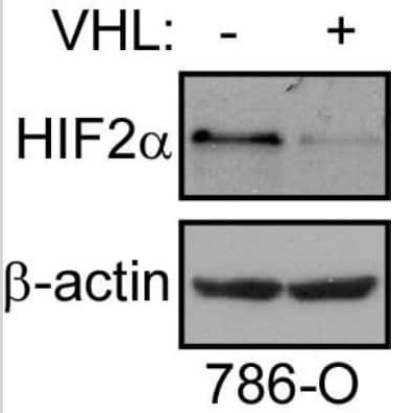
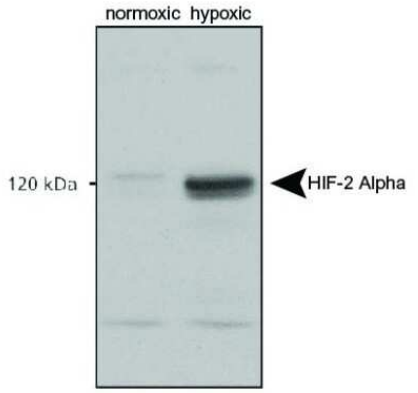
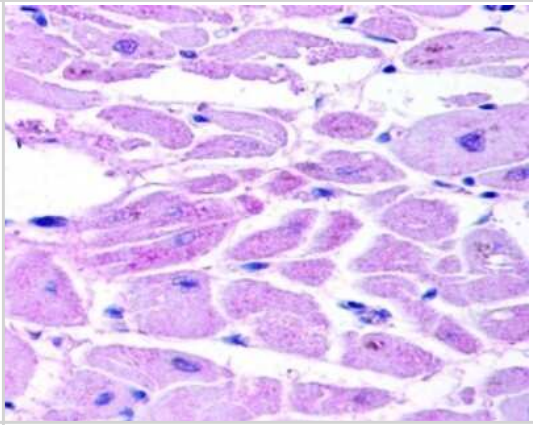
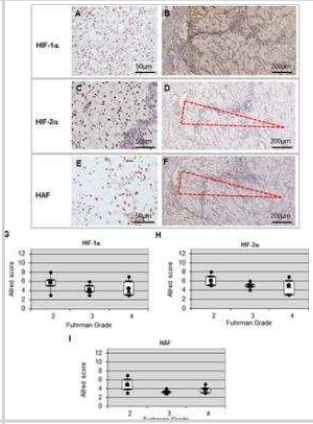
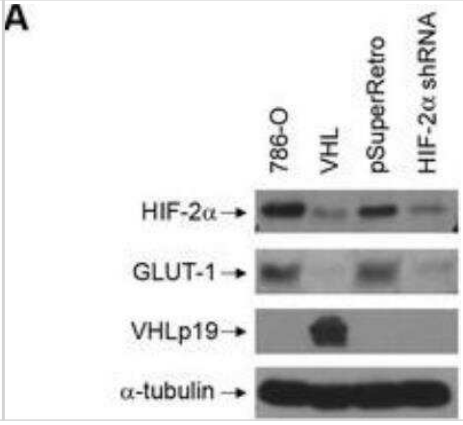
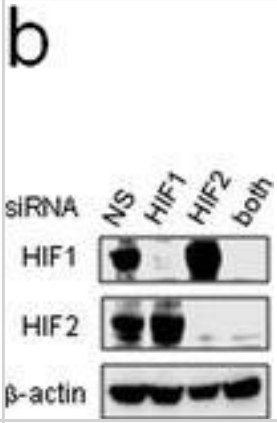
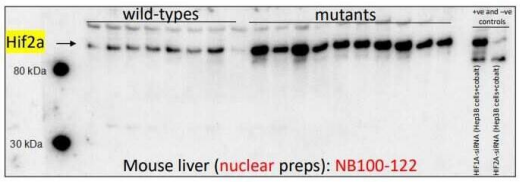
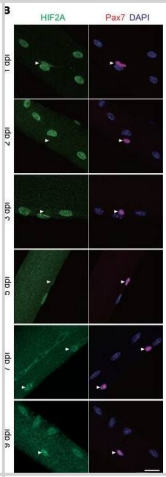
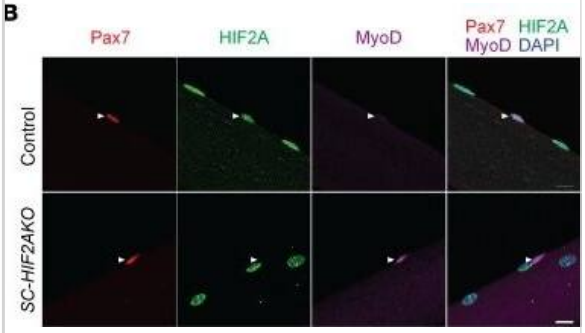
WNT11 is induced by hypoxia or hypoxic mimetics in different cell types. Immunoblot analyses of HeLa cells under normal air or hypoxia for 24 hrs. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/srep21520) licensed under a CC-BY license.	
HIF-1alpha is the predominant transcriptional regulator of WNT11 expression during hypoxia. EMSCs isolated from the indicated mouse genotypes were infected with lentivirus expressing GFP or Cre recombinase. Non-infected cells and GFP infected cells served as controls. Immunoblot analyses of EMSCs derived from the indicated genotypes treated with 0.1 mM DMOG for 24 hrs. Attenuated WNT11 expression in Hif-1alpha KO EMSCs (lenti-Cre infected Hif-1af/f). Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/srep21520) licensed under a CC-BY license.	
Formalin-fixed paraffin-embedded tissue sections of human placenta were probed for HIF-2 alpha/EPAS1 mRNA (ACD RNAScope Probe, catalog #410598; Fast Red chromogen, ACD catalog # 322750). Adjacent tissue section was processed for immunohistochemistry using Rabbit Polyclonal (Novus Biologicals catalog # NB100-122) at 1:100 dilution with one-hour incubation at room temperature followed by incubation with anti-rabbit IgG VisUCyte HRP Polymer Antibody (Catalog # VC003) and DAB chromogen (yellow-brown). Tissue was counterstained with hematoxylin (blue). Specific staining was localized to trophoblastic cells.	
Expression of hypoxia-inducible alpha subunits in normal and diseased lung tissue. HIF-2alpha expression are more evident in fibroblasts from idiopathic pulmonary fibrosis (d) than from lung tissue affected by other inflammatory conditions (i.e. chronic bronchitis, panel e) or normal lung tissue (f); as demonstrated by a higher proportion of positive fibroblasts (open arrows) than negative ones (solid black arrows). Image collected and cropped by CiteAb from the following publication (https://respiratory-research.biomedcentral.com/articles/10.1186/s12931-019-1100-4) licensed under a CC-BY license.	

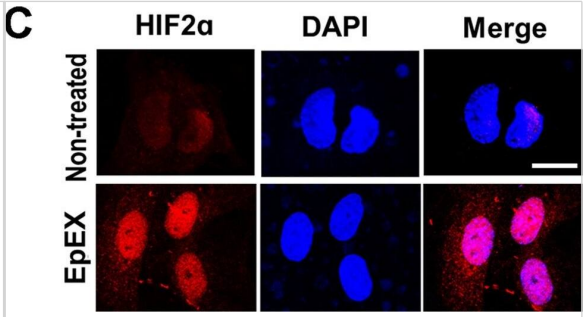
Image shows a specific band for HIF-2 alpha in 0.5 mg/mL of hypoxic HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Immunoblot validation of HIF2A and PHD2 KO clones using HIF2A (#NB100-122; dilution: 1/300), PHD2 (#NB100-137; dilution: 1/500). To blot HIFs factor cells were first pre-treated for 5 h with CoCl2 300 uM before protein extraction, a condition that promotes HIF factor accumulation. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/s41467-018-06988-3) licensed under a CC-BY license.	
HIF-2 alpha in human retinal and choroidal primary endothelia lysates using .. Image from verified customer review.	
HIF-2 alpha in MDA-MB-231 cell lysate (overexpression and endogenous samples) using . did not react to HIF-1 alpha overexpression. Image from verified customer review.	

Induced WNT11 expression with tumor hypoxia and WNT11 regulates tumor growth. Bevacizumab increased expression of HIF-1alpha and HIF-2alpha and WNT11. First 10 lanes are control tumors, and the last 10 lanes are tumors from bevacizumab-treated animals. Lysates from whole tissue and nuclei are indicated. Alpha -Tubulin, actin and lamin A/C are loading controls. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/srep21520) licensed under a CC-BY license.	
MDA-MB-231 cells were exposed to 20% or 1% O2 for 16 hours, and chromatin immunoprecipitation (ChIP) was performed with the indicated antibody (Ab). Primers flanking the HIF binding site were used for qPCR. ChIP image submitted by a verified customer review.	
Lane 1: Cobalt chloride treated COS7 nuclear extracts. Lane 2: Untreated COS7 nuclear extracts.	
786-O cells without or with VHL overexpression. Image from verified customer review.	

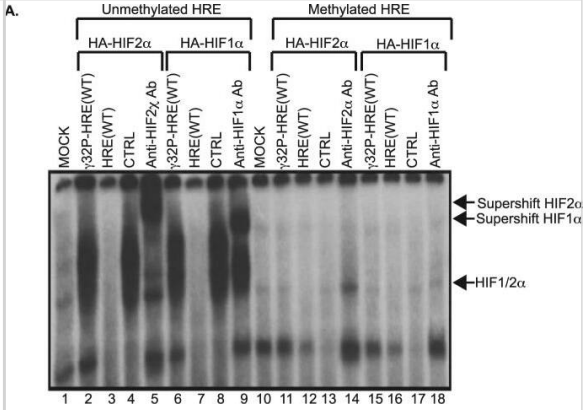
Analysis using the HRP conjugate of NB100-122. Detection of normoxic and hypoxic nuclear rat cell lysates.	
HIF-2 alpha immunoreactivity in human cardiac myocytes stained with NB100-122.	
Expression and correlation of 3 antibodies: HIF-1 alpha, HIF-2 alpha and HAF in ccRCC. (A, C & E) Positive nuclear staining to the 3 antibodies: HIF-1 alpha, HIF-2 alpha and HAF, in primary ccRCCs at high magnification (100X). (B, D & F) Heterogenous nuclear staining of 3 antibodies in a tumor at low magnification (20X). (G, H and I). Correlation of 3 antibodies with the Fuhrman grade. Citation: Ambrosetti D, Dufies M, Dadone B, Durand M, Borchiellini D, Amiel J, et al. (2018) The two glycolytic markers GLUT1 and MCT1 correlate with tumor grade and survival in clear-cell renal cell carcinoma. PLoS ONE 13(2): e0193477. https://doi.org/10.1371/journal.pone.0193477	
Reduction of HIF-2alpha levels leads to protection in UV-triggered apoptosis, but not for apoptosis caused by glucose and serum starvation in 786-O cells. Parental 786-O or those either stably expressing wild-type VHLp19 or stably infected with a control vector (pSuperRetro) or a pool of two HIF-2alpha shRNAs vectors [21] were grown to confluence and lysed. Cell alpha-tubulin.	

Sp1 and HIFs contribute to the synergistic activation of multiple genes in OVSAYO cells under SSH.b) Activation of multiple genes under hypoxia is dependent on HIFs. Western blotting is also shown for HIFs. Data shown are the mean (n = 3) ± SD.	
Western Blot: Rabbit Polyclonal HIF-2 alpha/EPAS1 Antibody [NB100-122] - Analysis of HIF-2 alpha/EPAS1 antibody on mouse liver nuclear extracts. Image from a verified customer review.	
Immunocytochemistry/ Immunofluorescence: HIF-2 alpha/EPAS1 Antibody - BSA Free [NB100-122] - Muscle repair following eccentric contraction–induced injury is concomitant with dynamic alterations of HIF2A & HIF1A expression in SCs.(A–C) Representative images of EDL myofibers from injured muscles at various time points (n >50 myofibers from 3 mice/group/time point) & stained for Pax7, DAPI, & EdU (A), HIF2A (B), or HIF1A (C). Scale bars: 20 µm. Arrowheads indicate SCs. (D) Number of Pax7+ SCs per myofiber at various time points. (E) Percentage of EdU+ SCs at various time points. (F) Percentage of HIF2A+ SCs at various time points. (G) Percentage of HIF1A+ SCs at various time points. Data represent the mean ± SEM. Image collected & cropped by CiteAb from the following publication (https://www.jci.org/articles/view/96208), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Immunocytochemistry/ Immunofluorescence: HIF-2 alpha/EPAS1 Antibody - BSA Free [NB100-122] - Genetic ablation of HIF2A in QSCs leads to transient activation, proliferation, & differentiation of SCs.(B) Representative images of myofibers from SC-HIF2AKO mice & control littermates (n >50 myofibers from 5 mice/group; 10 dpr). Immunofluorescence of Pax7 (red), HIF2A (green), MyoD (purple), & DAPI (blue) staining revealed HIF2A–MyoD+ & HIF2A+MyoD– SCs (arrowheads) in SC-HIF2AKO & control mice, respectively. Scale bar: 10 µm. Image collected & cropped by CiteAb from following publication (https://www.jci.org/articles/view/96208), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

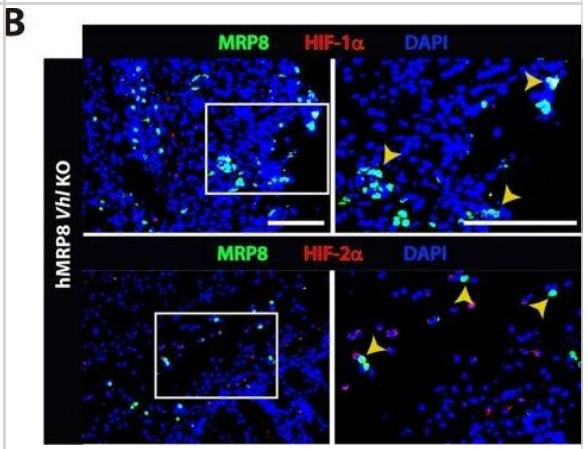
Immunocytochemistry/ Immunofluorescence: HIF-2 alpha/EPAS1 Antibody - BSA Free [NB100-122] - EpEX & EpCAM activate a STAT3-HIF2α signal for EpEX/EpCAM-mediated iPSC formation.(A) iPSCs were infected with two different EpCAM shRNAs (two clones, #1 & #2). The protein expressions of EpCAM & HIF2α were detected by Western blotting. (B) MEFs were stimulated by EpEX (1 μg/mL) at the indicated times. Nuclear-translocation was detected with a specific antibody against HIF2α (n = 3). (C) Immunofluorescence staining was performed to detect subcellular localization of HIF2α. Nuclei were stained with DAPI. Scale bar: 10 μm. (D) MEFs were treated with STAT3 inhibitor (WP1066, 10 μM), & then stimulated with EpEX for 30 min. The nuclear-translocation of HIF2α was detected by Western blotting with anti-HIF2α antibody (n = 3). (E) iPSC morphology was observed at day 20 after induction. Reprogramming of Oct4-GFP MEFs was induced by transfection of OSKM, OE + EpEX, & KE + EpEX with or without STAT3 inhibitor WP1066, or HIF2α shRNA (n = 3). Scale bar: 50 μm. Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/srep41852), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



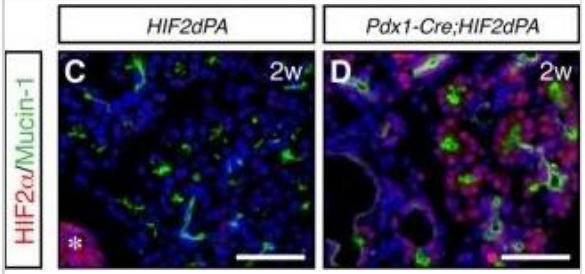
Gel Super Shift Assays: HIF-2 alpha/EPAS1 Antibody - BSA Free [NB100-122] - Manipulation of DNA methylation at HRE sequences alters HIF binding. (A) The Hypoxia Response Element (HRE) contains a CpG site that can be methylated which prevents HIF1 & HIF2 binding in vitro. EMSA of in vitro–translated HIF1 & HIF2 binding to 32P-labelled unmethylated & methylated HRE probes. Competition with 250X molar excess of unlabelled/unmethylated HRE probe (lanes 3, 7,12, 16) or unlabelled control HRE-free probe (lanes 4, 8, 13, 17). HIF1 & HIF2 complexes bound to unmethylated HRE or methylated HRE supershifted with anti-HIF1α (lanes 9 & 18) & anti-HIF2α (lanes 5 & 14). (B) RCC4-VHL cells were grown in normoxia (NT) or treated with 25 μM decitabine (5aza) or grown in hypoxia (1%) for 48 hrs & with the addition of 25 μM decitabine (1% + 5aza). QPCR was performed using primers specific to VEGF, uPAR, TGFα, GUS, U1AsnRNP1. Samples were normalised to relative expression of the housekeeping gene, ACTIN. Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/s41598-018-21524-5), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



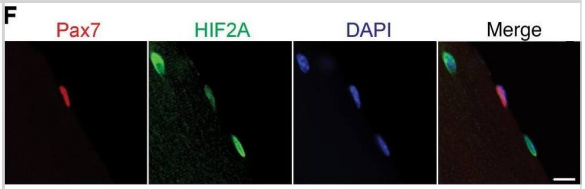
Immunocytochemistry/ Immunofluorescence: HIF-2 alpha/EPAS1 Antibody - BSA Free [NB100-122] - HIF-1α/2α expression in myeloid-specific KO mice targeting the HIF pathway. (A) Images of the colon of wild-type (WT), myeloid-specific Hif-1a KO (hMRP8 Hif-1a KO) or von Hippel Lindau (Vhl) KO (hMRP8 Vhl KO) mice, immunostained for MRP8 (green) & the DNA-binding regions of Hif-1a mRNA (red). Mice were fed with 5% DSS for 4 days prior to immunostaining analyses. Note that there were no MRP8-positive cells that were positive for Hif-1a mRNA in hMRP8 Hif-1a KO (middle column) mice, but we observed many cells that were double positive for MRP8 & Hif-1a mRNA in hMRP8 Vhl KO mice (right column). (B) Images of the colon of hMRP8 Vhl KO mice fed with 5% DSS as in A, immunostained for MRP8 (green) & HIF-1α (red, upper row) or HIF-2α (red, bottom row). DAPI-stained nuclei are shown in blue. White boxes in A & B indicate the regions magnified in the lower or right images, respectively. Yellow arrowheads in A & B indicate cells positive for both markers. Scale bars: 100 μm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29967068), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: HIF-2 alpha/EPAS1 Antibody - BSA Free [NB100-122] - HIF2α stabilization results in exocrine cell atrophy & expansion of duct-like tubular structures. (A) Body weight (left panel), pancreas weight (middle panel) & body/pancreas weight ratio (right panel) in Pdx1-Cre;HIF2dPA & control mice at 2 & 8 weeks of age. Data are presented as mean ± SD. (B) HIF2α accumulation in Pdx1-Cre;HIF2dPA analyzed by Western blot with anti-HA antibody. Two independent two-week-old control & mutant mice are shown. ß-actin protein was used for loading control. Full-length blots are presented in Supplementary Fig. 2. (D) Immunofluorescence analysis of HIF2α in two-week-old control pancreata. Endogenous HIF2α expression is observed in islets (marked by an white asterisk) but not in exocrine tissue. (D) Robust HIF2α accumulation in the pancreas of two-week-old Pdx1-Cre;HIF2dPA mice. Hematoxylin/Eosin-stained pancreatic sections from P0 (E,F), two- (I,J) & eight-week-old (M,N) Pdx1-Cre;HIF2dPA & control mice. Inset in N shows an area with adipose tissue in Pdx1-Cre;HIF2dPA pancreata. Immunofluorescence of amylase & KRT19 shows no differences between Pdx1-Cre;HIF2dPA & control mice at P0 (G,H). Duct-like tubular structures & loss of amylase immunoreactivity in two- (K,L) & eight-week-old (O,P) Pdx1-Cre;HIF2dPA mice compared to control mice. Note areas with normal acini in 8-week-old Pdx1-Cre;HIF2dPA mice (white asterisk in O). Insets in (H,L & P) show higher magnification pictures. DAPI staining is shown in blue in (C,D,G,H,K,L,O & P). Scale bars = 50 µm for (C,D); 100 µm for (E–P). ***P < 0.001. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30209343), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: HIF-2 alpha/EPAS1 Antibody - BSA Free [NB100-122] - QSCs are hypoxic in the niche & express HIF2A, but not HIF1A.(A) Timeline of in vivo pimonidazole labeling in SC-INTACT mice & representative confocal images of uninjured/resting EDL myofibers (n >50 myofibers from n = 3 mice) showing that nmGFP+ QSCs were pimonidazole+. Scale bars: 50 µm & 10 µm (insets). Inset images show that pimonidazole signals were relatively enriched in the cytoplasm of QSCs. Arrowheads indicate a QSC; asterisks indicate a myonucleus. (B) Percentage of pimonidazole+ QSCs. (C) Timeline of in vivo CCI-103F labeling in C57BL/6 mice & representative images of uninjured/resting EDL myofibers (n >50 myofibers from 3 mice) showing that nmGFP+ QSCs were CCI-103F+. Arrowheads indicate a QSC; asterisks indicate a myonucleus. Scale bar: 20 µm. (D) Percentage of CCI-103F+ QSCs. (E & F) Representative images of uninjured/resting EDL myofibers from C57BL/6 mice (n >50 myofibers from 6 mice/group) showing that most QSCs were HIF2A+, but HIF1A–. Scale bars: 10 µm. (G) Percentage of HIF1A+ & HIF2A+ QSCs. Data represent the mean ± SEM. Image collected & cropped by CiteAb from the following publication (https://www.jci.org/articles/view/96208), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Maesaka F, Nakai Y, Yoshida T et al. 5-Aminolevulinic Acid: A Novel Approach to Improving Radioresistance in Prostate Cancer. Cancers 2025-04-10 [PMID: 40282462]

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

Procedures

Western Blot protocol for HIF-2 alpha/EPAS1 Antibody (NB100-122)
General considerations for Western blot analysis of HIF-alpha proteins

1. HIF-2alpha is degraded under normoxic conditions and it is stabilized at O2 concentrations below 5% or with treatment using certain agents (CoCl2, DFO, etc.).

2. Positive and negative controls should always be run side by side in a Western blot to accurately identify the protein band upregulated in the hypoxic sample.

3. (HepG2 Hypoxic (CoCl2)/Normoxic Cell Lysate: NBP2-36451; HepG2 Hypoxic/Normoxic Cell Lysate: NBP2-36453).

4. To accurately compare treated and untreated samples and to ensure equal loading of samples the expression of a loading control should be evaluated.
(alpha Tubulin Antibody (DM1A): NB100-690)

5. The fully post-translationally modified form of HIF-2alpha is ~118 kDa, or larger.

6. HIF-2alpha may form a heterodimer with HIF-1beta. However, this is not typically seeing under denaturing conditions.

Western Blot Protocol

Materials

1x Laemmli Sample Buffer: 2% SDS, 2.5% 2-mercaptoethanol (bME), 25% glycerol, 0.01% bromophenol blue, 62.5 mM Tris HC, pH 6.8

1X Running Buffer: 25 mM Tris-base, 192 mM glycine, 0.1% SDS. Adjust to pH 8.3

1X Transfer buffer (wet): 25 mM Tris-base, 192 mM glycine, 20% methanol.

1X TBS

TBST (1X TBS with 0.1% Tween-20)

Blocking solution: TBST with 5% non-fat dry milk

Rabbit polyclonal anti-HIF-2 alpha primary antibody (NB100-122) in blocking solution (~1-2 ug/mL)

Methods

Whole-Cell Lysates

1. Load samples of treated and untreated cell lysates, 10-40 mg of total protein per lane on a 7.5% polyacrylamide gel (SDS-PAGE). Alternatively, gradient gels can be used for better resolution of lower molecular weight loading controls.

2. Resolve proteins by electrophoresis as required.

3. Transfer proteins to 0.45 mm PVDF membrane for 1 hour at 100V or equivalent.

4. Stain the blot using Ponceau S for 1-2 minutes to confirm efficient protein transfer onto the membrane.

5. Rinse the blot in distilled water to remove excess stain and mark the lanes and locations of molecular weight markers using a pencil.

6. Block the membrane using Blocking solution for 1 hour.

7. Dilute the rabbit anti-HIF-2 alpha primary antibody (NB100-122) in blocking solution (1-2 ug/ml) and incubate 1 hour at room temperature or overnight at 4oC.

8. Wash the membrane 3X 10 min in TBST.

9. Incubate in the appropriate diluted rabbit-IgG HRP-conjugated secondary antibody in blocking solution (as per manufacturer's instructions) for 1 hour at room temperature.

10. Wash the membrane 3X10 min in TBST.

11. Apply the detection reagent of choice in accordance with the manufacturer's instructions (e.g., ECL, ECL Plus). Image blot.

Immunocytochemistry/Immunofluorescence protocol for HIF-2 alpha/EPAS1 Antibody (NB100-122)
HIF-2 alpha/EPAS1 Antibody:
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

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Products Related to NB100-122

NB800-PC26	COS-7 Nuclear Hypoxic Induced Cell Lysate
NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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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