Product Datasheet

EGLN1/PHD2 Antibody - BSA Free
NB100-137

Unit Size: 0.1 mg

Store at 4C. Do not freeze.

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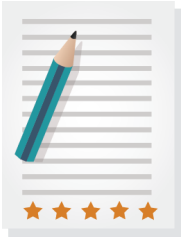
Updated 4/13/2025 v.20.1



NB100-137

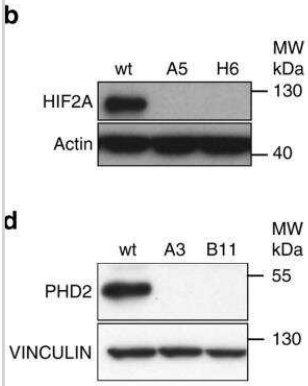
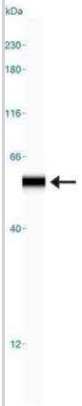
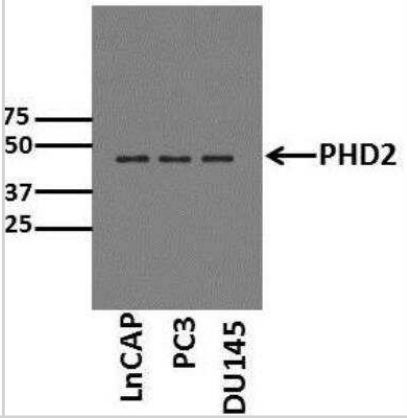
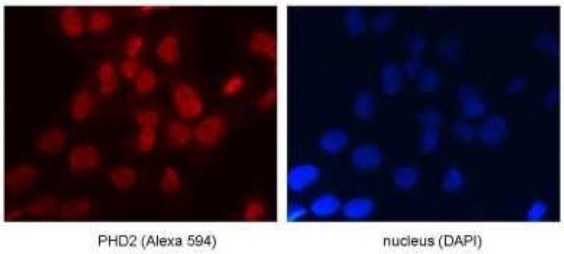
EGLN1/PHD2 Antibody - BSA Free

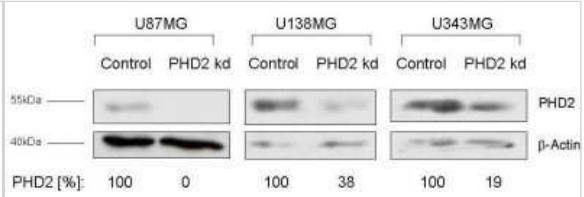
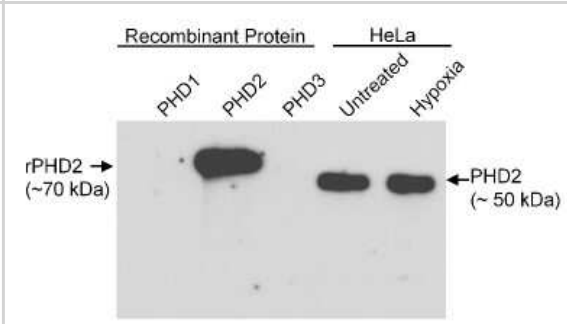
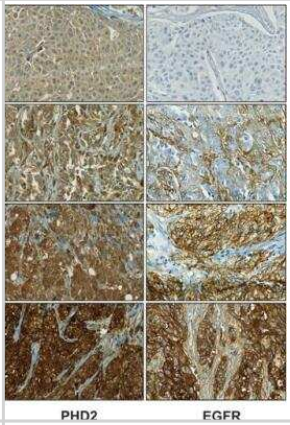
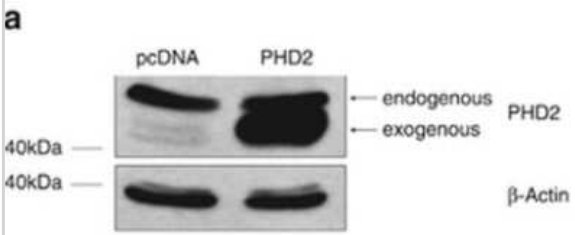
Product Information	
Unit Size	0.1 mg
Concentration	1 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.09% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	Tris-Citrate/Phosphate (pH 7 to 8)
Target Molecular Weight	46 kDa

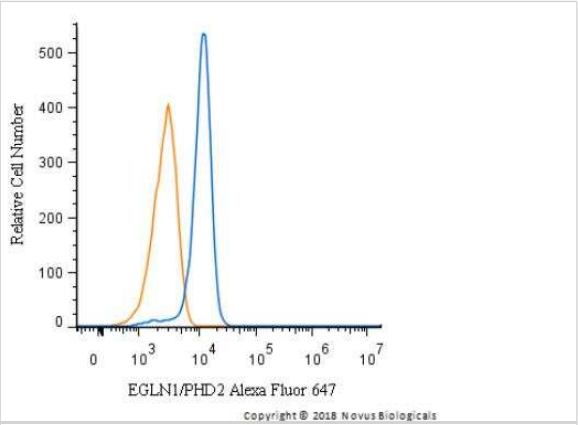
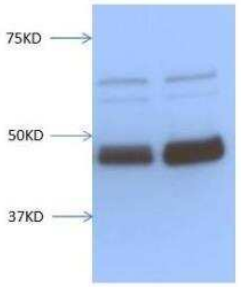
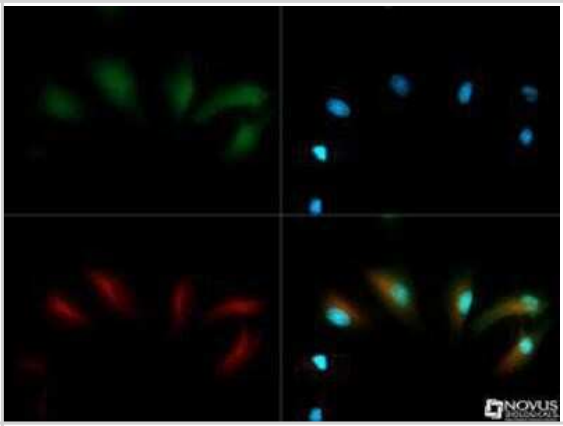
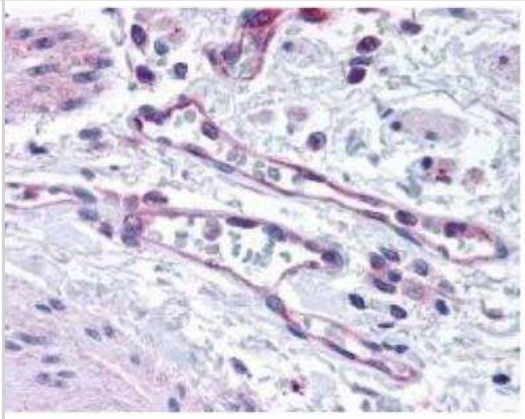
Product Description	
Host	Rabbit
Gene ID	54583
Gene Symbol	EGLN1
Species	Human, Mouse, Rat, Primate
Reactivity Notes	Results for use of this EGLN1/PHD2 antibody have been mixed in Rat with success in Western blot analysis and immunofluorescence on Rat endothelial cells and negative results with PC12 cells. Mouse reactivity reported in scientific literature (PMID: 25578858). Rat reactivity reported in scientific literature (PMID: 25635047). Primate reactivity reported in scientific literature (PMID: 25974097)
Immunogen	The epitope recognized by this EGLN1/PHD2 antibody maps to a region between residues 1 and 50 of human PHD2/HIF Prolyl Hydroxylase 2 using the numbering given in entry NP_071334.1 (GeneID 54583).

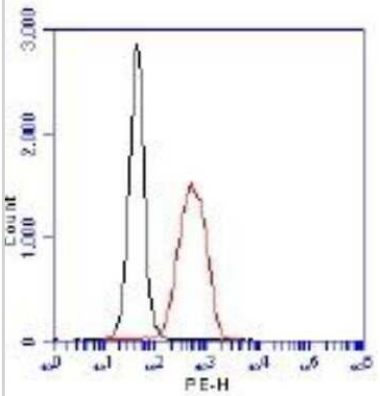
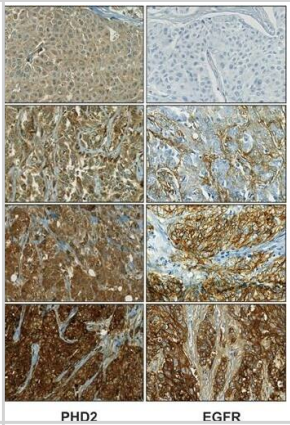
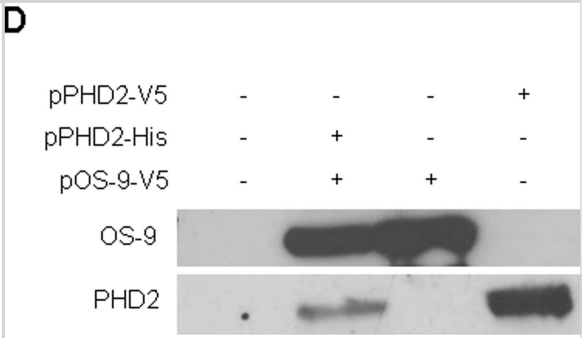
Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 1:500 - 1:2500, Simple Western 1:500, Flow Cytometry 3.0 mcg/mL, Immunohistochemistry 1:10 - 1:500, Immunocytochemistry/ Immunofluorescence 1:50, Immunoprecipitation, Immunohistochemistry-Paraffin 1:10 - 1:500, Knockout Validated, Knockdown Validated
Application Notes	This EGLN1/PHD2 antibody is useful for Flow Cytometry, Immunocytochemistry/Immunofluorescence, Western Blot, and Immunohistochemistry-paraffin embedded sections. In ICC/IF, cytoplamic and nuclear staining was observed in HeLa cells. Immunoprecipitation was reported in scientific literature. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in Hypoxic HeLa lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:500, apparent MW was 57 kDa. 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

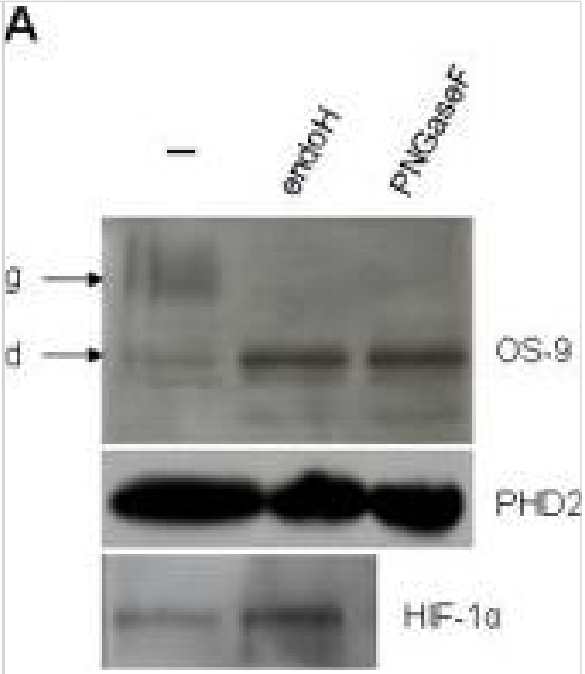
Western Blot: EGLN1/PHD2 Antibody [NB100-137] - Immunoblot validation of HIF2A and PHD2 KO clones using HIF2A (#NB100-122; dilution: 1/300), PHD2 (#NB100-137; dilution: 1/500). To blot HIFs factor cells were first pre-treated for 5 h with CoCl2 300 uM before protein extraction, a condition that promotes 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.	
Simple Western: EGLN1/PHD2 Antibody [NB100-137] - Simple Western lane view shows a specific band for PHD2/HIF Prolyl Hydroxylase 2 in 0.5 mg/mL of hypoxic HeLa cell lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Western Blot: EGLN1/PHD2 Antibody [NB100-137] - Analysis of PHD2 expression in human prostate cancer cell lines. Western blot image submitted by a verified customer review.	
Immunocytochemistry/Immunofluorescence: EGLN1/PHD2 Antibody [NB100-137] - Staining of endogenous PHD2 in U2OS cells. ICC/IF image submitted by a verified customer review.	

Western Blot: EGLN1/PHD2 Antibody [NB100-137] - Detection of PHD2 in human glioblastoma cell lysates. Image submitted by a verified customer review.	
Western Blot: EGLN1/PHD2 Antibody [NB100-137] - Detection of human PHD2 by Western blot. Recombinant epitope-tagged PHD1, PHD2 or PHD3 (10 ng/lane) or whole cell lysate from HeLa cells. EGLN1/PHD2 antibody used at 1 ug/mL. Detection by chemiluminescence.	
Immunohistochemistry: EGLN1/PHD2 Antibody [NB100-137] - EGLN1/PHD2 and EGFR expression levels positively correlate in breast cancer. Processed tissue microarrays of breast cancer biopsies from 313 patients were stained with EGLN1/PHD2 and EGFR antibodies. Four representative immunohistochemistries of human breast cancer with low and high expression of EGLN1/PHD2 and EGFR are shown. Magnification 10x. Image collected and cropped by CiteAb from the following publication (https://www.oncotarget.com/article/14241/text/) licensed under a CC-BY license.	
Western Blot: EGLN1/PHD2 Antibody [NB100-137] - Exogenous PHD2 has no impact on the gene expression of HIF-1alpha and HIF-2alpha. U87MG cells were transfected with empty vector (pcDNA) or PHD2 plasmid. Twenty-four hours after transfection, PHD2 was detected by immunoblotting. beta-Actin was used as loading control. The results are representative for three independent experiments. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/cddis2014295), licensed under a CC-BY license.	

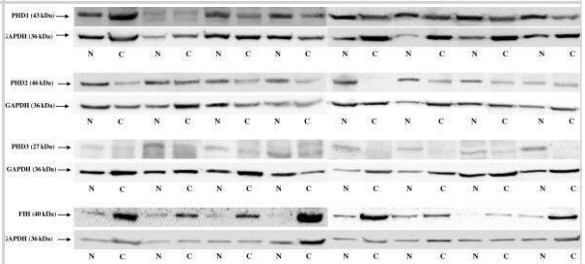
Flow Cytometry: EGLN1/PHD2 Antibody [NB100-137] - An intracellular stain was performed on Jurkat cells with NB100-137AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.	
Western Blot: EGLN1/PHD2 Antibody [NB100-137] - Analysis of PHD2 in normoxia (lane 1) or hypoxia (lane 2) treated SK-N-BE(2) cells using anti-EGLN1/PHD2 antibody. Western blot image submitted by a verified customer review.	
Immunocytochemistry/Immunofluorescence: EGLN1/PHD2 Antibody [NB100-137] - Staining of PHD2 in HeLa cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).	
Immunohistochemistry: EGLN1/PHD2 Antibody [NB100-137] - Staining of lung vascular endothelium with EGLN1/PHD2 antibody. Image at 40X.	

Flow Cytometry: EGLN1/PHD2 Antibody [NB100-137] - Detection of PHD2 in Jurkat cells. One million Jurkat cells were fixed, permeabilized, and stained with 3.0 ug/mL anti-EGLN1/PHD2 antibody in a 150 uL reaction. Isotype control (black), anti-MLL1 (red).	
PHD2 and EGFR expression levels positively correlate in breast cancerProcessed tissue microarrays of breast cancer biopsies from 313 patients were stained with PHD2 and EGFR antibodies (cf Materials and methods). Four representative immunohistochemistries of human breast cancer with low and high expression of PHD2 and EGFR are shown. Magnification 10x.	
Initial characterization of the OS-9 protein.(A) OS-9 expression in various human cell lines. Equal protein amounts of total cell lysates were used for SDS-PAGE & subsequent Western blotting. For each cell line, two independent samples are shown. Endogenous OS-9 was detected with a polyclonal antibody raised against a peptide corresponding to amino acids 600–667 of isoform 1 of OS-9. (B) Protein stability assay of endogenous OS-9. U2OS cells were treated with the translational inhibitor cycloheximide (100 µM). At indicated time points, whole cell lysates were analysed by immunoblotting. (C) Effect of hypoxia on OS-9 expression. For hypoxia, UT-7 cells were exposed to 1% O2 for 24 h prior to Western blot analysis. To determine any influence of HIF-1α on OS-9 expression under normoxia, cells were incubated with the prolyl hydroxylase inhibitor DMOG (0.5 mM) for 24 h. (D) Protein interaction between OS-9 & PHD2 in vitro. For co-immunoprecipitation, U2OS cells were transiently co-transfected with the plasmids pOS-9-V5 & pPHD2-His, lysed in NP40 buffer, & subjected to immunoisolation with anti-V5 antibody recognizing OS-9 by its V5-tag. OS-9 & its associated proteins were separated by SDS-PAGE & analyzed by Western blot (lane 2). As controls, samples of untransfected (lane 1) cells or cells transfected with a single plasmid (lanes 3–4) were loaded. Representative Western blots are shown for each subfigure. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/21559462), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

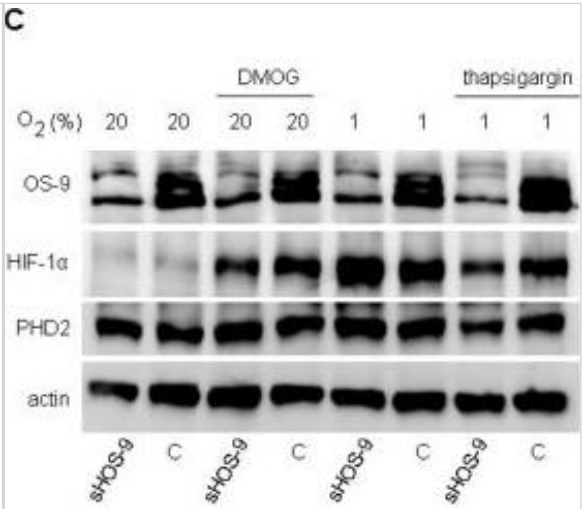
Cellular localization of OS-9 & PHD2.(A) A lectin gel-shift assay was conducted to test for glycosylated proteins. Total cell lysates of U2OS cells were incubated in the presence or absence of the endoglycosidases EndoH & PNGaseF for 6 h at 37°C. Digest products were separated on a reducing 10% SDS-PAGE gel which contained concanavalin A co-polymerized in the top layer of the separating gel to retard mobility of glycosylated proteins [56]. Glycosylated OS-9 is indicated as ‘g’, deglycosylated OS-9 as ‘d’. (B) Detection of OS-9 & PHD2 in the nuclear fraction. HEK293 cells with & without transfection of the plasmid pcDNA3-OS-9 were separated into nuclear fraction (N) & postnuclear supernatant (PS), the latter containing cytoplasm & organelles. Western blot analysis included BiP, GAPDH & lamin A as typical marker proteins for the ER, the cytoplasm & the nucleus, respectively. (C) Detection of OS-9 & PHD2 in the cytoplasm. HEK293 cells were co-transfected with pcDNA3-OS-9 & pPHD2-V5. For hypoxia, cells were exposed to 3% O2 for 4 h. Cells were treated with 50 µg/ml digitonin & centrifuged to obtain a cytoplasmic (C) & an organelle fraction (O) & subjected to immunoblotting. (D) Isolation of cellular endomembranes. HEK293 cells were lysed mechanically by several passages through a 30½G needle. The postnuclear supernatant was processed further by ultra-centrifugation to separate the organelles (O) from the cytosol (C). High salt treatment (1 M KCl) of the organelle fraction produced a wash fraction (W) that contained dissociated peripheral membrane proteins. For immunoblot analysis of subcellular fractionations, cell aliquots were normalized for cell number prior to loading (B–D). Representative Western blots are shown for each subfigure. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/21559462), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



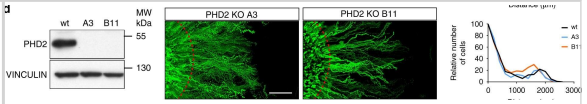
Western Blot: EGLN1/PHD2 Antibody [NB100-137] - Representative picture of western blot in histopathologically unchanged tissue (N) & primary cancerous tissue (C) from patients with CRC. Immunodetection of bands was performed with Rp anti- PHD1, - PHD2, - PHD3 & - FIH Ab, followed by incubation with goat anti-rabbit HRP-conjugated Ab. The membrane was stripped & incubated with Rp anti-GAPDH Ab, followed by incubation with goat anti-rabbit HRP-conjugated Ab. Bands were revealed using SuperSignal West Femto Chemiluminescent Substrate, Thermo Fisher Scientific (Rockford, IL) & Biospectrum® Imaging System 500, UVP Ltd. (Upland, CA). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/24195777), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



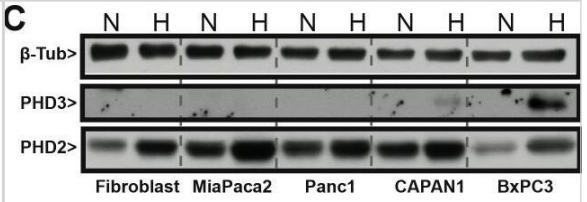
Western Blot: EGLN1/PHD2 Antibody [NB100-137] - OS-9 shows no effect on regulation of HIF-1α.Total cell lysates were used for SDS-PAGE & subsequent Western blotting. To generate nearly anoxic conditions, cells were exposed to an oxygen consuming chemical system or to 1% or 3% O2 for 4 h to generate hypoxia. (A) U2OS, HeLa & Hep3B cells were transiently transfected with the plasmid pOS-9-V5 48 h prior to the experiment. Lamin A & actin were used as loading controls. (B) U2OS cells were subjected to ER stress by incubation either with tunicamycin (1 µg/ml) or thapsigargin (0.5 µg/ml) for 20 h. To detect HIF-1α under normoxia, cells were treated with DMOG (1 mM) for 4 h. A sample of DMSO-only treated cells was loaded to exclude unspecific side effects of the solvent. (C) U2OS cells were transduced with lentiviral construct pLKO.1-shRNA-OS-9 (shOS-9) mediating a stable knockdown of OS-9 expression. Control cells (c) were transduced with plasmid pLKO.1-puro. Representative Western blots are shown for each subfigure. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/21559462), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



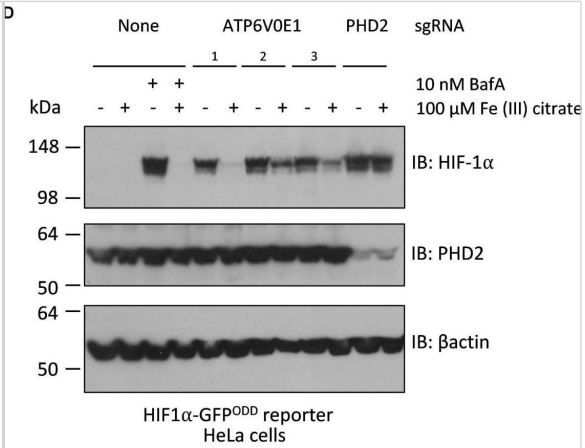
Western Blot: EGLN1/PHD2 Antibody [NB100-137] - Oxygen chemotaxis is independent of the PHD/HIF pathway. a–dHIF1A, HIF2A, HIF1A +HIF2A & PHD2 CRISPR/Cas9 KO clones characterisation, respectively, regarding O2-directed migration. Left panels: immunoblot validation of HIF1A, HIF2A, HIF1A+HIF2A & PHD2 KO clones. To blot HIFs factors (a, b, c), cells were first pre-treated for 5 h with CoCl2 300 µM before protein extraction, a condition that promotes HIF factors accumulation (cf. Supplementary Fig. 8f). Middle panels: cell trajectories under confinement. Red dashed lines indicate the border of the cell cluster at 0 h. Right panels: relative distribution of MCF10A KO clones versus wt cells at the edge of the cluster at 48 h. These experiments demonstrate that HIF factors deletion does not prevent aerotaxis. e, f Tracks & redistribution of wt & PHD2 KO clone silenced for PHD3 (siPHD3) or not (siCTR). g Tracks & redistribution of MCF10A cells treated with DMOG (50 µM) or CoCl2 (50 µM) to inhibit PHDs, or with vehicle only (DMSO). These experiments demonstrate that PHDs do not participate in O2-sensing during aerotaxis. Confinement was applied for 48 h (a–g). Scale bars, 500 µm Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30382089), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



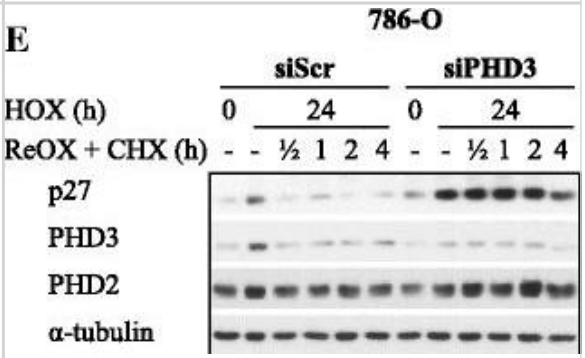
Western Blot: EGLN1/PHD2 Antibody [NB100-137] - PHD3 expression correlates with a mesenchymal-like morphology in pancreatic ductal adenocarcinoma cell lines.NHF-1 (Fibroblast) MiaPaca2, Panc1, CAPAN1 & BxPC3 cells were harvested for RNA & protein following 24 hours exposure to normoxia (21% O2) or hypoxia (1% O2). (A) Phase-contrast images at 10× magnification were taken of MiaPaca2 (mesenchymal-like) morphology & BxPC3 cells (differentiated, epithelial morphology) under normoxic conditions. (B) PHD3 mRNA expression was determined by qRT-PCR & graphed relative to BxPC3 in normoxia. All samples were normalized to 18S rRNA & graphed as expression relative to BxPC3-Vec Normoxia (lane 1). n=3, error bars =1 S.D. (C) Whole cell lysate was resolved by SDS-PAGE & blotted for β-tubulin PHD3 & PHD2. N=normoxia, H=hypoxia (1% O2). Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0083021), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: EGLN1/PHD2 Antibody [NB100-137] - Iron supplementation restores HIF-1α levels to normal following ATP6V0E1 inhibition in HeLa cells. (A) Schematic diagram of the multimeric V-ATPase complex. (B) Chemical inhibition of V-ATPase by 10 nM BafA treatment for 24 h, increased HIF-1α levels in HIFα-GFPODD reporter cells. Treatment with 100 μM Fe (III) citrate significantly reduced the elevated HIF-1α levels associated with loss of ATP6V0E1 (1 × 106 cells per sample harvested & analyzed; N = 2). (C) Knock-down of ATP6V0E1 subunit with three different CRISPR-Cas9 guides resulted in significant upregulation of HIF-1α levels in HIFα-GFPODD reporter cells. Co-treating cells with 100 μM Fe (III) citrate led to a reduction in HIF-1α levels across the three ATP6V0E1 depleted cells. phd2 was knocked down as a control & treatment with 100 μM Fe (III) citrate did not result in reduction of HIF-1α levels (1 × 106 cells per sample harvested & analyzed; N = 2). FACs plot shown is a representative image of two biological repeats performed. (D) Immunoblot analysis for HIF-1α & PHD2 levels in HIFα-GFPODD reporter cells with either ATP6V0E1 or PHD2 depleted or treated with 10 nM BafA. The cells were treated with 100 μM Fe (III) citrate for 24 h. β actin was used as a control. Results validated findings observed by flow cytometry, whereby HIF-1α levels were upregulated following ATP6V0E1 knock-down or inhibition & levels were re-normalized upon Fe (III) citrate treatment. Treatment of Fe (III) citrate in PHD2 depleted cells did not alter HIF-1α levels. All experiments were performed in biological duplicate. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32984302), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: EGLN1/PHD2 Antibody [NB100-137] - PHD3 depletion stabilizes hypoxic p27 expression by increasing p27 half-life. a Cell cycle arrest at G0 & subsequent release shows an increase of p27 expression in siPHD3 exposed cells. b Quantification for p27 expression under PHD3 depletion at indicated time points after cell cycle release in HeLa & 786-O cells. Asterisk indicates significant difference ($p < 0,05$; $n = 3$). c Cell cycle arrest at G0 & inhibition of protein synthesis with cycloheximide indicate increased p27 stability in PHD3 depleted HeLa cells. d Quantification of p27 expression using siPHD3 or control at indicated time points. Four independent experiments (± SEM) are shown ($p < 0,05$; $n = 4$). e Analysis of p27 stability in 786–0 cells by cycloheximide chase during reoxygenation after 24 h hypoxia demonstrates markedly increased half-life of p27 upon PHD3 depletion Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26223520), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Lam YT, Lecce L, Yuen SC, Wise SG et Al. Androgens Ameliorate Impaired Ischemia-Induced Neovascularization Due to Aging in Male Mice Endocrinology 2019-03-05 [PMID: 30830222]

Lassi Luomala, Kalle Mattila, Paula Vainio, Harry Nisén, Teijo Pellinen, Jouni Lohi, Teemu D. Laajala, Petrus Järvinen, Anna□Riina Koskenniemi, Panu Jaakkola, Tuomas Mirtti Low nuclear expression of HIF □hydroxylases PHD2 / EGLN1 and PHD3 / EGLN3 are associated with poor recurrence□free survival in clear cell renal cell carcinoma Cancer Medicine 2024-02-24 [PMID: 38400673]

Kozlova N, Wottawa M, Katschinski DM et al. Hypoxia-inducible factor prolyl hydroxylase 2 (PHD2) is a direct regulator of epidermal growth factor receptor (EGFR) signaling in breast cancer. Oncotarget 2017-02-07 [PMID: 28038470]

C Iacobini, M Vitale, G Pugliese, S Menini Normalizing HIF-1alpha Signaling Improves Cellular Glucose Metabolism and Blocks the Pathological Pathways of Hyperglycemic Damage Biomedicines, 2021-09-02;9(9):. 2021-09-02 [PMID: 34572324]

Miikkulainen P, Hogel H, Seyednasrollah F et al. Hypoxia-inducible factor (HIF)-prolyl hydroxylase 3 (PHD3) maintains high HIF2A mRNA levels in clear cell renal cell carcinoma. J. Biol. Chem. 2019-01-07 [PMID: 30617181]

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More publications at http://www.novusbio.com/NB100-137

Procedures

Immunohistochemistry Protocol for PHD2/HIF Prolyl Hydroxylase 2 Antibody (NB100-137)

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 Celsius.

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 Celsius 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).

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