Product Datasheet

EGLN1/PHD2 Antibody
NB100-137

Unit Size: 0.1 mg

Store at 4C. Do not freeze.

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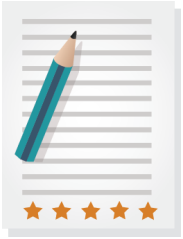
Updated 12/20/2023 v.20.1



NB100-137

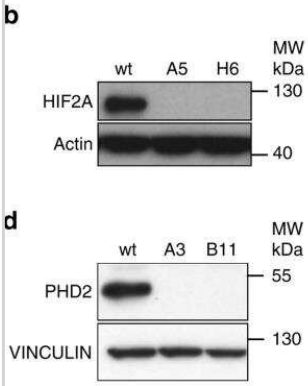
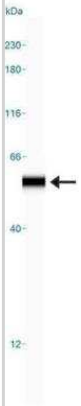
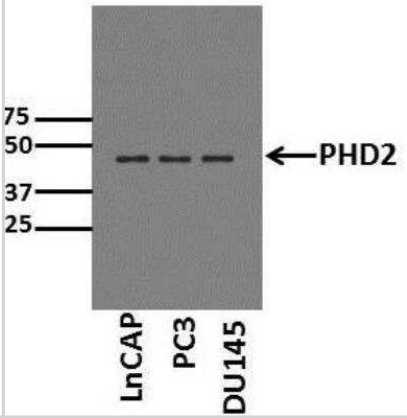
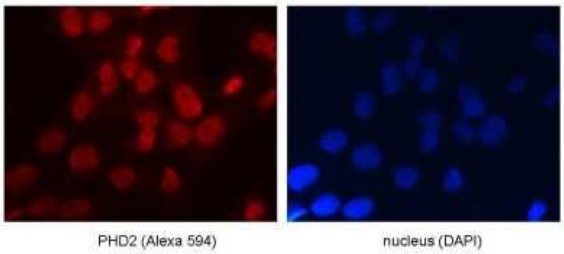
EGLN1/PHD2 Antibody

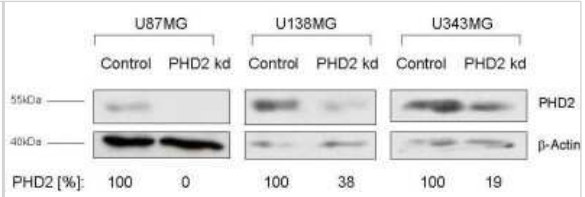
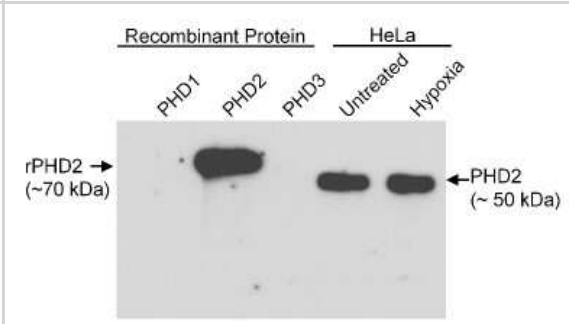
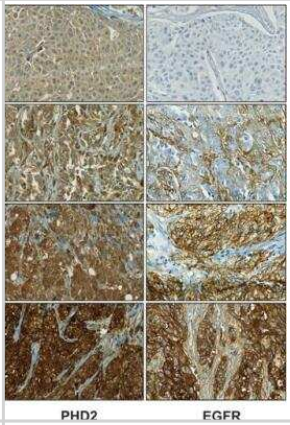
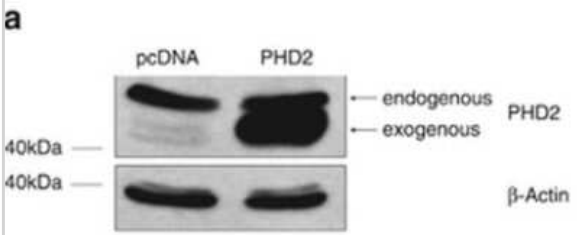
Product Information	
Unit Size	0.1 mg
Concentration	1 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.09% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	Tris-Citrate/Phosphate (pH 7 to 8)
Target Molecular Weight	46 kDa

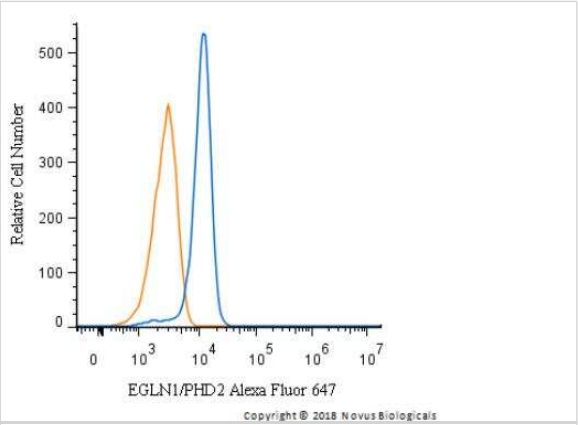
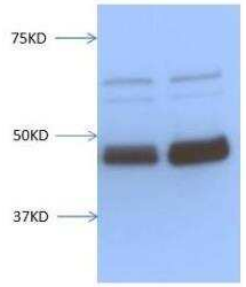
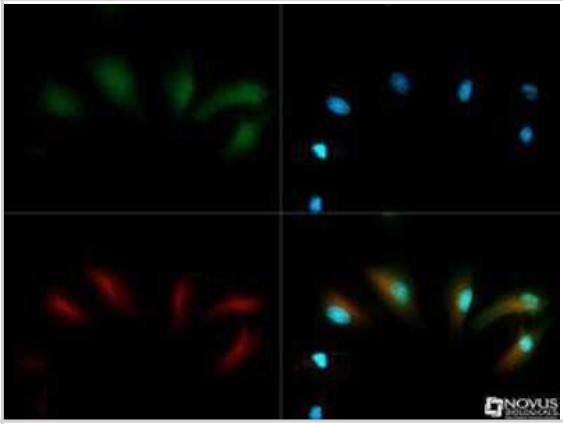
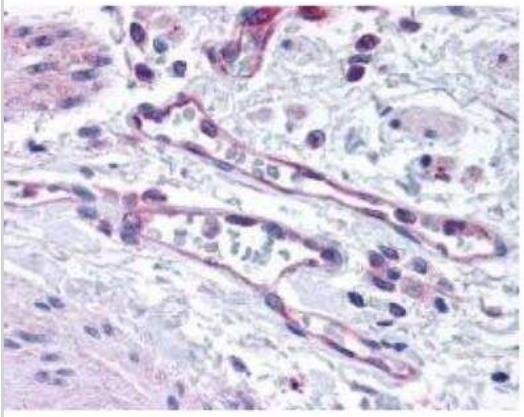
Product Description	
Host	Rabbit
Gene ID	54583
Gene Symbol	EGLN1
Species	Human, Mouse, Rat, Primate
Reactivity Notes	Results for use of this EGLN1/PHD2 antibody have been mixed in Rat with success in Western blot analysis and immunofluorescence on Rat endothelial cells and negative results with PC12 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. 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.

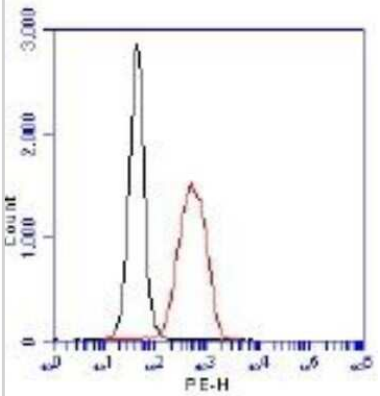
Images

Knockout Validated: 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.	

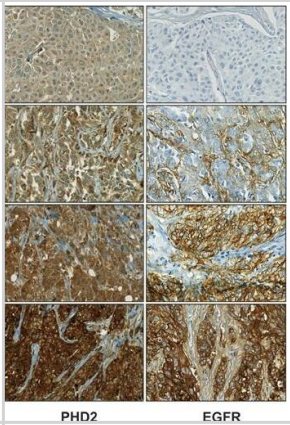
Knockdown Validated: EGLN1/PHD2 Antibody [NB100-137] - Detection of PHD2 in human glioblastoma cell lysates. Image submitted by a verified customer review.	
Western Blot: EGLN1/PHD2 Antibody [NB100-137] - Detection of human PHD2 by Western blot. Recombinant epitope-tagged PHD1, PHD2 or PHD3 (10 ng/lane) 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.	

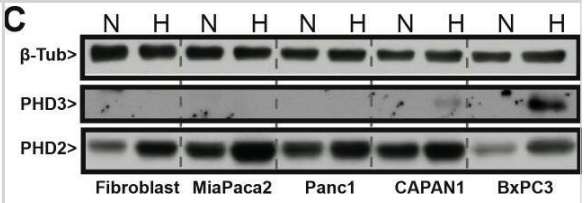
Flow Cytometry: EGLN1/PHD2 Antibody [NB100-137] - Detection of PHD2 in Jurkat cells. One million Jurkat cells were fixed, permeabilized, and stained with 3.0 ug/mL anti-EGLN1/PHD2 antibody in a 150 uL reaction. Isotype control (black), anti-MLL1 (red).



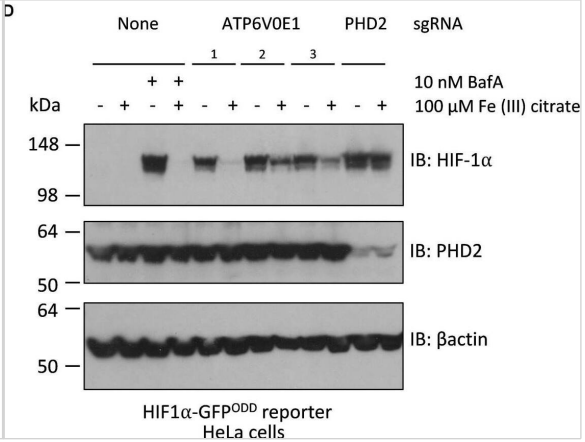
PHD2 and EGFR expression levels positively correlate in breast cancerProcessed tissue microarrays of breast cancer biopsies from 313 patients were stained with PHD2 and EGFR antibodies (cf Materials and methods). Four representative immunohistochemistries of human breast cancer with low and high expression of PHD2 and EGFR are shown. Magnification 10x.

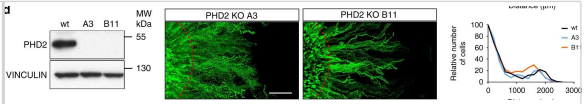
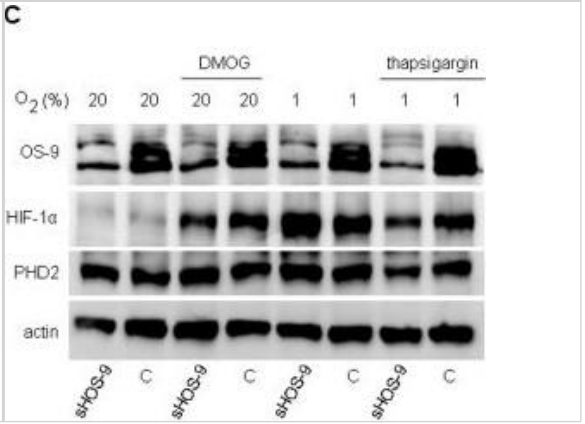


PHD3 expression correlates with a mesenchymal-like morphology in pancreatic ductal adenocarcinoma cell lines.NHF-1 (Fibroblast) MiaPaca2, Panc1, CAPAN1 and BxPC3 cells were harvested for RNA and protein following 24 hours exposure to normoxia (21% O2) or hypoxia (1% O2). (C) Whole cell lysate was resolved by SDS-PAGE and blotted for beta-tubulin PHD3 and PHD2. N = normoxia, H = hypoxia (1% O2). Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0083021), licensed under a CC-BY licence.



Iron supplementation restores HIF-1alpha levels to normal following ATP6V0E1 inhibition in HeLa cells. (D) Immunoblot analysis for HIF-1alpha and PHD2 levels in HIFalpha-GFPODD reporter cells with either ATP6V0E1 or PHD2 depleted or treated with 10 nM BafA. The cells were treated with 100 uM Fe (III) citrate for 24 h. beta actin was used as a control. Results validated findings observed by flow cytometry, whereby HIF-1alpha levels were upregulated following ATP6V0E1 knock-down or inhibition and levels were re-normalized upon Fe (III) citrate treatment. Treatment of Fe (III) citrate in PHD2 depleted cells did not alter HIF-1alpha levels. All experiments were performed in biological duplicate. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32984302), licensed under a CC-BY licence.



Rapamycin reduces the accumulation of BMAL1 in Per2 knockout miceA. As shown in the left panel, tissue samples from Per2 knockout mice (mPER−/−) depict robust accumulation of nuclear BMAL1 (arrow) compared to control littermates (arrowhead)(*** p<0.001). Administration of Rapamycin reduces the accumulation of BMAL1 in the epidermis of mPer−/− mice (arrowhead) compared to mPer−/− mice receiving vehicle alone (** p<0.01) to levels comparable to wild-type mice receiving vehicle alone (ns: p>0.05). Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/27285754), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Characterization of CIP2A expression in a panel of normal human melanocytes (NHM) and melanoma cell lines. (A) Immunoblot analysis and (B) quantification of protein expression levels of CIP2A (C) CIP2A mRNA expression measured by qRT-PCR. NHM1 line was used as calibrator. (D) Cytoplasmic and nuclear expression of CIP2A in normal melanocytes, primary and metastatic melanoma cell lines assessed by immunoblot analysis and (E) by immunohistochemical staining. CIP2A, cancerous inhibitor of protein phosphatase 2A. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/25663244), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Publications

Dey A, Prabhudesai S, Zhang Y et al. Cystathione ?-synthase regulates HIF-1? stability through persulfidation of PHD2 Science Advances 2020-07-03 [PMID: 32937467] (WB)

Marinaccio C, Suraneni P, Celik H et al. LKB1/STK11 Is a Tumor Suppressor in the Progression of Myeloproliferative Neoplasms Cancer Discovery 2021-06-01 [PMID: 33579786] (WB, B/N)

Sallais J, Park C, Alahari S et al. HIF1 inhibitor acriflavine rescues early-onset preeclampsia phenotype in mice lacking placental prolyl hydroxylase domain protein-2 JCI insight 2022-10-13 [PMID: 36227697] (WB, Mouse)

Wang F, Yu H, Huang S et al. Jian-Pi-Yi-Shen Regulates EPO and Iron Recycling Protein Expressions in Anemic Rats with Chronic Kidney Disease: Accumulation of Hypoxia Inducible Factor-2 alpha via ERK Signaling Evid Based Complement Alternat Med 2020-11-12 [PMID: 33178327]

Bhute V, Harte J, Houghton J, Maxwell P Mannose binding lectin is hydroxylated by collagen prolyl-4-hydroxylase and inhibited by some PHD inhibitors Kidney360 2022-04-04 [PMID: 35368589]

Schlegel C, Liu K, Spring B et al. Decreased expression of hypoxia-inducible factor 1 alpha (HIF-1 alpha) in cord blood monocytes under anoxia Pediatric research 2022-07-29 [PMID: 35906309] (WB, Human)

Jian CB, Yu XE, Gao HD et al. Liposomal PHD2 Inhibitors and the Enhanced Efficacy in Stabilizing HIF-1alpha Nanomaterials (Basel, Switzerland) 2022-01-03 [PMID: 35010112] (WB, Human)

Chen T, Zhou Q, et al. miR-17/20 Controls Prolyl Hydroxylase 2 (PHD2)/Hypoxia-Inducible Factor 1 (HIF1) to Regulate Pulmonary Artery Smooth Muscle Cell Proliferation. J Am Heart Assoc 2016-12-05 [PMID: 27919930] (WB, Mouse)

Sundaramurthi, H, Roche, S L Et al. Selective Histone Deacetylase 6 Inhibitors Restore Cone Photoreceptor Vision or Outer Segment Morphology in Zebrafish and Mouse Models of Retinal Blindness. Front Cell Dev Biol 2020-09-29 [PMID: 32984302] (IP, Mouse)

Li J, Lu X, Wei L Et al. PHD2 attenuates high-glucose-induced blood retinal barrier breakdown in human retinal microvascular endothelial cells by regulating the Hif-1 alpha/VEGF pathway Inflammation research : official journal of the European Histamine Research Society ... [et al.] 2021-11-13 [PMID: 34773469] (WB, Human)

Kumar P, Verma V, Mohania D Et al. Leukemia associated RUNX1T1 gene reduced proliferation and invasiveness of glioblastoma cells Journal of cellular biochemistry 2021-08-09 [PMID: 34369622]

Mei H, Wu N, Huang X et al. Possible mechanisms by which silkworm faeces extract ameliorates adenine-induced renal anaemia in rats J Ethnopharmacol 2020-10-03 [PMID: 33022342] (WB, Rat)

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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Products Related to NB100-137

NBL1-10153	EGLN1/PHD2 Overexpression Lysate
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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