Product Datasheet

EGLN1/PHD2 Antibody - BSA Free
NB100-2219SS

Unit Size: 0.025 ml

Store at 4C. Do not freeze.

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Reviews: 3 Publications: 42

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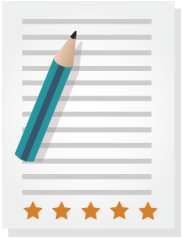
Updated 10/23/2024 v.20.1



NB100-2219SS

EGLN1/PHD2 Antibody - BSA Free

Product Information

Unit Size	0.025 ml
Concentration	1 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	43 kDa

Product Description

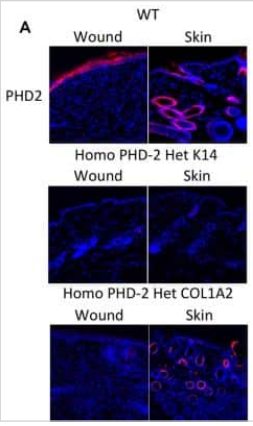
Host	Rabbit
Gene ID	54583
Gene Symbol	EGLN1
Species	Human, Mouse, Rat
Immunogen	This EGLN1/PHD2 antibody was developed against a synthetic peptide made to an internal portion of mouse PHD2/HIF Prolyl Hydroxylase 2 (between residues 300-400). [Uniprot: Q91YE3]

Product Application Details

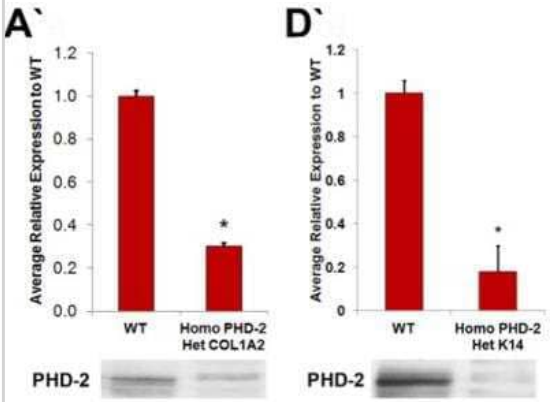
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 2 ug/mL, Immunohistochemistry 2.5 - 5.0 ug/mL, Immunocytochemistry/ Immunofluorescence 1:50 - 1:500, Immunoprecipitation 1:10 - 1:500, Immunohistochemistry-Paraffin 2.5 - 5.0 ug/mL, Knockout Validated reported in scientific literature (PMID 24695462), Knockdown Validated
Application Notes	In Western blot a band is seen ~43 kDa representing HIF Prolyl Hydroxylase 2. There is also a non-specific band of similar intensity at ~75 kDa.

Images

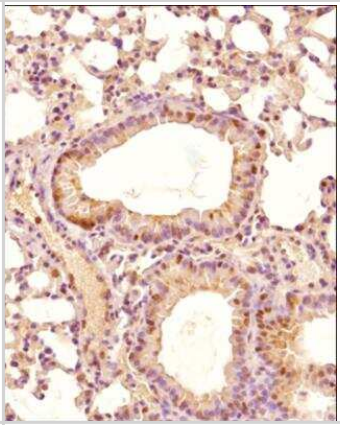
Immunohistochemistry: EGLN1/PHD2 Antibody [NB100-2219] - IHC and Immunofluorescence of wounded tissue and normal unwounded skin. Representative images for immunofluorescent staining of PHD-2 on healed wounded skin and adjacent normal unwounded skin of wild type, heterozygous K14-Cre/homozygous floxed PHD-2, and heterozygous Col1 alpha2-Cre-ER/homozygous floxed PHD-2 mice at 400x magnification. Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0093373), licensed under a CC-BY license.



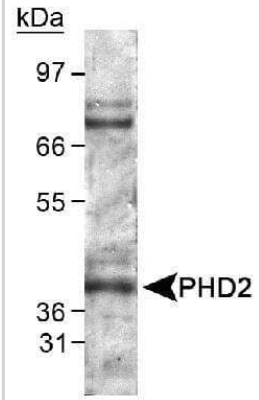
Knockdown Validated: EGLN1/PHD2 Antibody [NB100-2219] - In vitro analysis of PHD-2 knockout and protein quantification. Western blot data for PHD-2 knockout in fibroblasts of heterozygous Col1 alpha2-Cre-ER/homozygous floxed PHD-2 mice compared to wild type mice (*p<0.05). D') Western blot data for PHD-2 knockout in keratinocytes of heterozygous K14-Cre/homozygous floxed PHD-2 mice compared to wild type mice (*p<0.05). Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0093373), licensed under a CC-BY license.



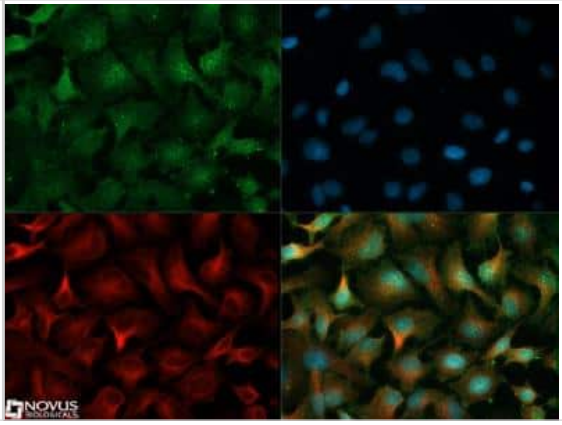
Immunohistochemistry-Paraffin: EGLN1/PHD2 Antibody [NB100-2219] - Analysis of an FFPE mouse lung section using 1:200 dilution of EGLN1/PHD2 antibody. The staining was developed using HRP conjugated anti-rabbit secondary antibody and DAB reagent. The antibody generated a specific staining in the cytoplasm and nuclei of alveolar as well as bronchiolar epithelial cells. Cytoplasmic staining was observed in almost all cells while the nuclear positivity was seen in a subset of cells only.



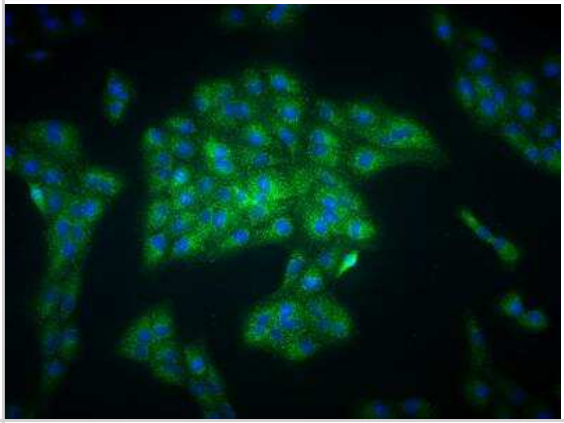
Western Blot: EGLN1/PHD2 Antibody [NB100-2219] - Detection of EGLN1/PHD2 in mouse kidney lysate. ECL exposure, 20 seconds.



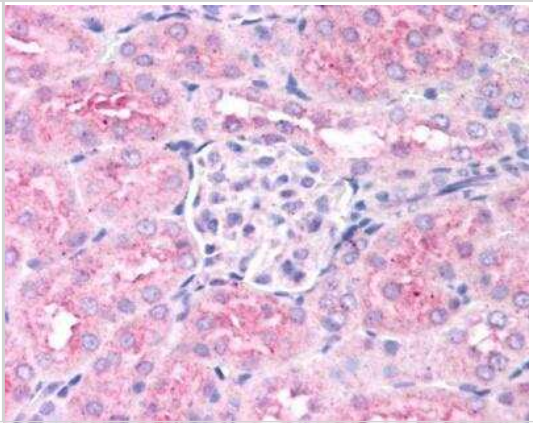
Immunocytochemistry/Immunofluorescence: EGLN1/PHD2 Antibody [NB100-2219] - EGLN1/PHD2 antibody at 1:500 in HeLa cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).



Immunocytochemistry/Immunofluorescence: EGLN1/PHD2 Antibody [NB100-2219] - Analysis of EGLN1/PHD2 in ARPE-19 cells using anti-PHD2 antibody. ICC/IF image submitted by a veriifed customer review.



Immunohistochemistry: EGLN1/PHD2 Antibody [NB100-2219] - Staining of renal tubular epithelium in mouse using NB100-2219 at 2.5 ug/mL.



Publications

Nasyrov E, Nolan KA, Wenger RH et al. The neuronal oxygen-sensing pathway controls postnatal vascularization of the murine brain FASEB J. 2019-08-30 [PMID: 31469589]

Megan E. Vaughan, Martina Wallace, Michal K. Handzlik, Alanna B. Chan, Christian M. Metallo, Katja A. Lamia Cryptochromes Suppress HIF1α in Muscles iScience 2020-07-03 [PMID: 32683313]

Anna Laitakari, Joona Tapio, Kari A. Mäkelä, Karl-Heinz Herzig, Franziska Dengler, Helena Gylling, Gail Walkinshaw, Johanna Myllyharju, Elitsa Y. Dimova, Raisa Serpi, Peppi Koivunen HIF-P4H-2 inhibition enhances intestinal fructose metabolism and induces thermogenesis protecting against NAFLD Journal of Molecular Medicine (Berlin, Germany) 2020-04-15 [PMID: 32296880]

Zhang, Y;Pan, YD;Zheng, WY;Li, HY;Zhu, MZ;Ou Yang, WJ;Qian, Y;Turecki, G;Mechawar, N;Zhu, XH; Enhancing HIF-1?-P2X2 signaling in dorsal raphe serotonergic neurons promotes psychological resilience Redox biology 2023-12-21 [PMID: 38150991]

Noonan ML, Ni P, Solis E et al. Osteocyte Egln1/Phd2 links oxygen sensing and biomineralization via FGF23 Bone Research 2023-01-18 [PMID: 36650133] (Western Blot)

Sun L, Wu C, Ming J et al. EGLN1 induces tumorigenesis and radioresistance in nasopharyngeal carcinoma by promoting ubiquitination of p53 in a hydroxylase-dependent manner Journal of Cancer 2022-04-08 [PMID: 35517429] (IHC-P, Human)

Elamaa H, Kaakinen M, NAtynki M et al. PHD2 deletion in endothelial or arterial smooth muscle cells reveals vascular cell type-specific responses in pulmonary hypertension and fibrosis Angiogenesis 2022-01-08 [PMID: 34997404] (WB, Mouse)

ROning T, Magga J, Laitakari A et al. Activation of the hypoxia response pathway protects against age-induced cardiac hypertrophy Journal of molecular and cellular cardiology 2021-12-14 [PMID: 34919895] (WB, Mouse)

Ju S, Lim L, Wi K et al. LRP5 Regulates HIF-1 alpha Stability via Interaction with PHD2 in Ischemic Myocardium International Journal of Molecular Sciences 2021-06-19 [PMID: 34205318] (IP, Rat)

van Kuijk K, Demandt JAF, Perales-PatOn J et al. DEFICIENCY OF MYELOID PHD PROTEINS AGGRAVATES ATHEROGENESIS VIA MACROPHAGE APOPTOSIS AND PARACRINE FIBROTIC SIGNALING: Atherogenic effects of myeloid PHD knockdown Cardiovascular research 2021-04-26 [PMID: 33913468] (IF/IHC, Human)

Laitakari A, Huttunen R, Kuvaja P et al. Systemic long-term inactivation of hypoxia-inducible factor prolyl 4-hydroxylase 2 ameliorates aging-induced changes in mice without affecting their life span FASEB J. 2020-02-25 [PMID: 32100354] (WB, Mouse)

Kim S, Jo CH, Kim GH Effects of empagliflozin on nondiabetic salt-sensitive hypertension in uninephrectomized rats Hypertens. Res. 2019-09-19 [PMID: 31537914] (WB, Rat)

More publications at http://www.novusbio.com/NB100-2219

Procedures

Western Blot Protocol for PHD2/HIF Prolyl Hydroxylase 2 Antibody (NB100-2219)

Western Blot Protocol

1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 40 ug of total protein per lane.

2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.

3. Rinse membrane with dH2O and then stain the blot using ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.

4. Rinse the blot in TBS for approximately 5 minutes.

5. Block the membrane using 5% non-fat dry milk + 1% BSA in TBS for 2 hours at room temperature (RT).

6. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.

7. Dilute the rabbit anti-PHD2 (murine) primary antibody (NB 100-2219) in blocking buffer and incubate 1 hour at RT.

8. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.

9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) and incubate 1 hour at RT.

10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each (this step can be repeated as required to reduce background).

11. Apply the detection reagent of choice in accordance with the manufacturer's instructions (we used BioFX Super Plus ECL). Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

IHC-FFPE sections

I. Deparaffinization:

Western Blot Protocol

1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 40 ug of total protein per lane.

2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.

3. Rinse membrane with dH2O and then stain the blot using ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.

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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. 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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Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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