Product Datasheet

EGLN1/PHD2 Antibody
NB100-137SS

Unit Size: 0.025 ml

Store at 4C. Do not freeze.

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NB100-137SS

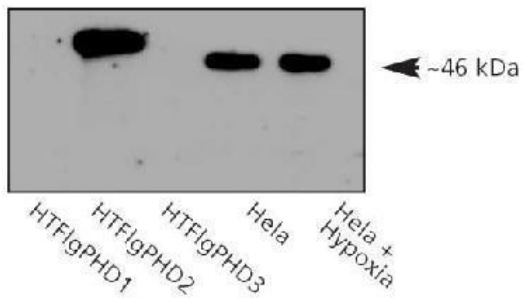
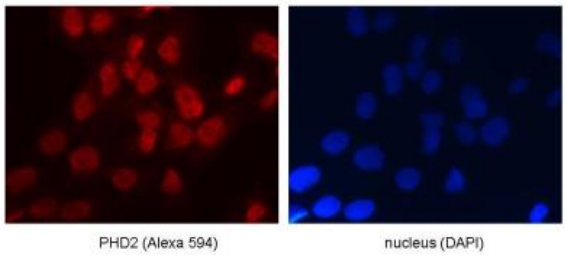
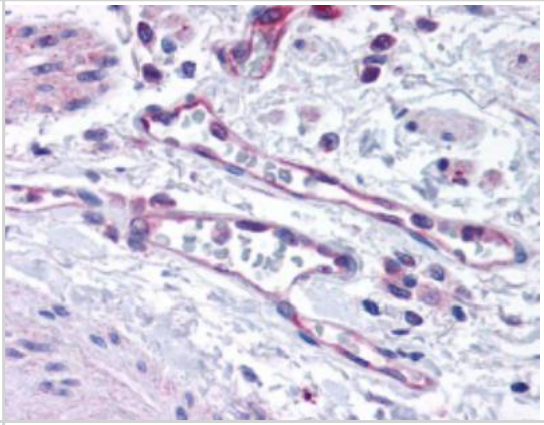
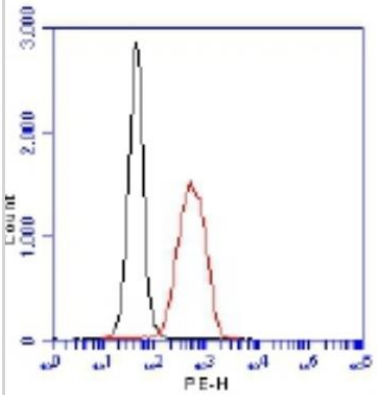
EGLN1/PHD2 Antibody

Product Information	
Unit Size	0.025 ml
Concentration	1 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.09% Sodium Azide
Purity	Immunogen affinity purified
Buffer	Tris-citrate/phosphate, pH 7-8
Target Molecular Weight	46 kDa

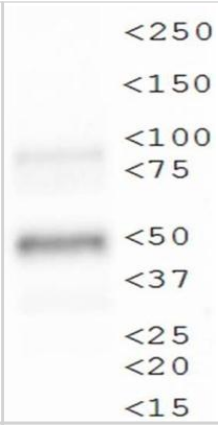
Product Description	
Host	Rabbit
Gene ID	54583
Gene Symbol	EGLN1
Species	Human, Mouse, Rat, Rat (Negative)
Species Reactivity	Human. This antibody does not appear to work in Mouse. Results have been mixed in Rat with success in western analysis and immunofluorescence on Rat endothelial cells and negative results with PC12 cells. Expected reactivity with Rabbit, Orangutan, Rhesus Monkey, Gorilla based on 100% sequence identity.Mouse reactivity reported in scientific literature (PMID: 25578858) Rat reactivity reported in scientific literature (PMID: 25635047)
Immunogen	The epitope recognized by this 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
Recommended Dilutions	Flow Cytometry 3.0 mcg/ml, Immunocytochemistry/Immunofluorescence 1:50, Immunohistochemistry 1:10-1:500, Immunohistochemistry-Paraffin 1:10-1:500, Immunoprecipitation, Western Blot 1:500-1:2500, Simple Western 1:500
Application Notes	This 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.

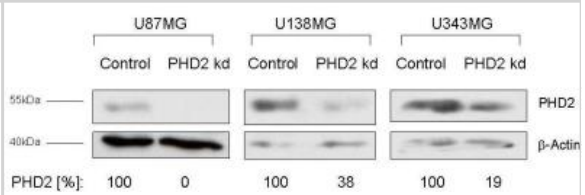
Images

Western Blot: PHD2/HIF Prolyl Hydroxylase 2 Antibody [NB100-137] - Western Blot analysis of human PHD2, using NB100-137. Samples: Recombinant FLAG-His-PHD1, PHD2 or PHD3 (10ng/lane) and whole cell lysate from HeLa cells.	
Immunocytochemistry/Immunofluorescence: PHD2/HIF Prolyl Hydroxylase 2 Antibody [NB100-137] - Immunofluorescence of endogenous PHD2 in U2OS cells. Image from verified customer review.	
Immunohistochemistry: PHD2/HIF Prolyl Hydroxylase 2 Antibody [NB100-137] - Staining of Vascular Endothelium 40X in Lung using NB100-137.	
Flow Cytometry: PHD2/HIF Prolyl Hydroxylase 2 Antibody [NB100-137] - Flow cytometric detection of PHD2. 1 million Jurkat cells were fixed, permeabilized, and stained with 3.0 ug/ml anti-PHD2 NB100-137 in a 150 ul reaction. Isotype control (black), anti-MLL1 (red).	

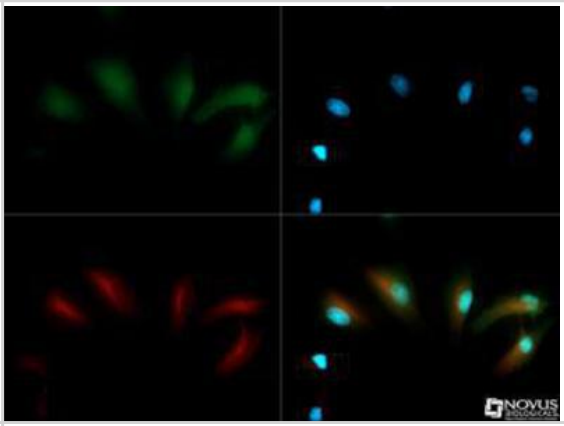
Western Blot: PHD2/HIF Prolyl Hydroxylase 2 Antibody [NB100-137] - PHD2 antibody was tested in Hep3B cell lysate.



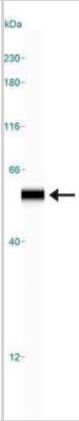
Western Blot: PHD2/HIF Prolyl Hydroxylase 2 Antibody [NB100-137] - Western blot detection of PHD2 human glioblastoma cells. Image from verified customer review.



Immunocytochemistry/Immunofluorescence: HIF Prolyl Hydroxylase 2 Antibody [NB100-137] - PHD2 antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



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 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



Publications

Hsiao HW, Hsu TS, Liu WH et al. Deltex1 antagonizes HIF-1alpha and sustains the stability of regulatory T cells in vivo Nat Commun. 2015 Feb 20 [PMID: 25695215] (WB, Human, Mouse)

Li J, Yuan W, Jiang S et al. Prolyl-4-hydroxylase Domain Protein 2 Controls NF-kB/p65 Transactivation and Enhances the Catabolic Effects of Inflammatory Cytokines on cells of the Nucleus Pulposus J. Biol. Chem. 2015 Jan 29 [PMID: 25635047] (ICC/IF, Rat)

Details:
EGLN1/PHD2 antibody used for ICC-IF analysis of nucleus pulposus/NP cells (rat) following 1 and 4 hours of TNF-alpha treatment (FIGURE 6C). The immunoassay involved - 4% paraformaldehyde fixation, 10 minutes of permeablization with 0.2% Triton X-100 -PBS, blocking with 5% FBS-PBS, 4C ON incubation of cells with 1:200 dilution of primary antibody or isotype IgG, detection with Alexa Fluor 594-conjugated anti-rabbit secondary antibody used at 1:200 dilution with 1 hour incubation.

Vogler M, Zieseniss A, Hesse AR et al. Pre- and post-conditional inhibition of prolyl-4-hydroxylase domain enzymes protects the heart from an ischemic insult Pflugers Arch. 2015 Jan 13 [PMID: 25578858] (IHC-P, Mouse)

Details:
EGLN1/PHD2 antibody used at 1:10,000 dilution for IHC-P application on heart sections from C57BL/6 mice after 1, 6, and 24 h of treatment (40-fold magnification) with ICA, a specific PHD inhibitor 2-(1-chloro-4-hydroxyisoquinoline-3-carboxamido) acetate - two doses of 40 mg/kg BW ICA or vehicle i.p., either 6 and 1 h before myocardial infarction or 1 and 5 h after myocardial infarction (Fig 3).

Henze At, Garvalov Bk, Seidel S et al. Loss of PHD3 allows tumours to overcome hypoxic growth inhibition and sustain proliferation through EGFR. Nat Commun. 2014 Nov 25 [PMID: 25420773]

Botting KJ, McMillen IC, Forbes H, Nyengaard JR. Chronic hypoxemia in late gestation decreases cardiomyocyte number but does not change expression of hypoxia-responsive genes. J Am Heart Assoc. 2014 Aug 02 [PMID: 25085511] (WB)

Sun W, Jelkmann W, Depping R. Prolyl-4-hydroxylase 2 enhances hypoxia-induced glioblastoma cell death by regulating the gene expression of hypoxia-inducible factor-a. Cell Death Dis 2014 Jul 11 [PMID: 25010988] (WB, Human)

Details:
PHD2/HIF Prolyl Hydroxylase 2 antibody used for WB at 1 : 500 dilution on 30-60ug lysates of U87MG, U138MG and U343MG cells (Figure 1 - wild type control and PHD2 knock-down cells), and in U87MG cells transfected with empty vector pcDNA or PHD2 plasmid (Figure 7 a- endogenous and over-expressed PHD2).

Lindholm ME, Fischer H, Poellinger L et al. Negative regulation of HIF in skeletal muscle of elite endurance athletes - a tentative mechanism promoting oxidative metabolism Am. J. Physiol. Regul. Integr. Comp. Physiol. 2014 Jun 04 [PMID: 24898836] (WB, Human)

Details:
PHD2/HIF Prolyl Hydroxylase 2 antibody used for WB on human biopsy samples (vastus lateralis muscle obtained via percutaneous needle technique) in experiments involving endurance-trained /elite cyclists-triathletes and moderately active men (Figures 2B, 4)

Caprara V, Silvia S, Garrafa E et al. Endothelin-1 regulates hypoxia-inducible factor-1a and-2a stability through prolyl hydroxylase domain 2 inhibition in human lymphatic endothelial cells. Life sciences 3/6/2014 [PMID: 24607784] (WB, Human)

Place TL, Nauseef JT, Peterson MK et al. Prolyl-4-Hydroxylase 3 (PHD3) Expression Is Downregulated during Epithelial-to-Mesenchymal Transition. PLoS One 2013 Dec 18 [PMID: 24367580] (WB, Human)

Rawluszko AA, Bujnicka KE, Horbacka K et al. Expression and DNA methylation levels of prolyl hydroxylases PHD1, PHD2, PHD3 and asparaginyl hydroxylase FIH in colorectal cancer. BMC Cancer. 2013 Nov 6 [PMID: 24195777] (WB, Human)

Zhu Q, Hu J, Han WQ et al. Silencing of HIF Prolyl-Hydroxylase 2 Gene in the Renal Medulla Attenuates Salt-Sensitive Hypertension in Dahl S Rats. Am J Hypertens. 2013 Nov 4 [PMID: 24190904] (WB, Rat)

Bordoli MR, Stiehl DP, Borsig L et al. Prolyl-4-hydroxylase PHD2- and hypoxia-inducible factor 2-dependent regulation of amphiregulin contributes to breast tumorigenesis. Oncogene. 2011 Feb 3 [PMID: 20856199] (IHC-P, Human)

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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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 guarantee, please visit www.novusbio.com/guarantee.