Product Datasheet

EGLN1/PHD2 Antibody
NB100-2219SS

Unit Size: 0.025 ml

Store at 4C. Do not freeze.

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NB100-2219SS

EGLN1/PHD2 Antibody

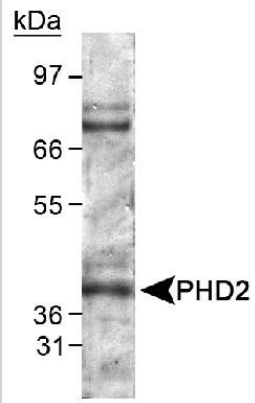
Product Information	
Unit Size	0.025 ml
Concentration	1 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	43 kDa

Product Description	
Host	Rabbit
Gene ID	54583
Gene Symbol	EGLN1
Species	Human, Mouse
Species Reactivity	Human and Mouse. Predicted to react with rat based on 100% sequence homology.
Immunogen	A synthetic peptide made to an internal portion of mouse PHD2/HIF Prolyl Hydroxylase 2 (between residues 300-400). [UniProt# Q91YE3]

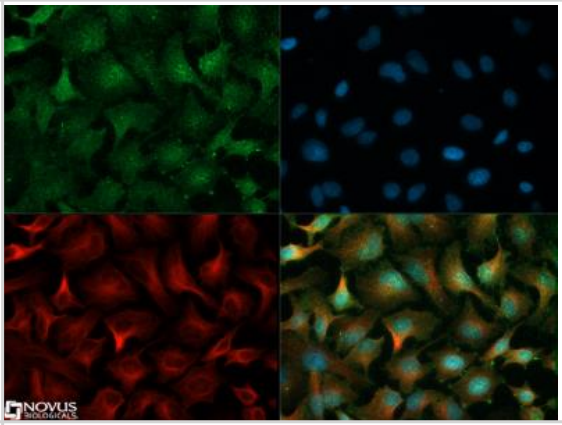
Product Application Details	
Applications	Western Blot, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Immunocytochemistry/Immunofluorescence 1:50-1:500, Immunohistochemistry 2.5 ug/ml, Immunohistochemistry-Paraffin 2.5 ug/ml, Immunoprecipitation 1:10-1:500, Western Blot 2 ug/ml
Application Notes	This HIF Prolyl Hydroxylase 2 antibody is useful for Western blot, Immunocytochemistry/Immunofluorescence, Immunohistochemistry-Paraffin embedded sections and Immunoprecipitation. 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

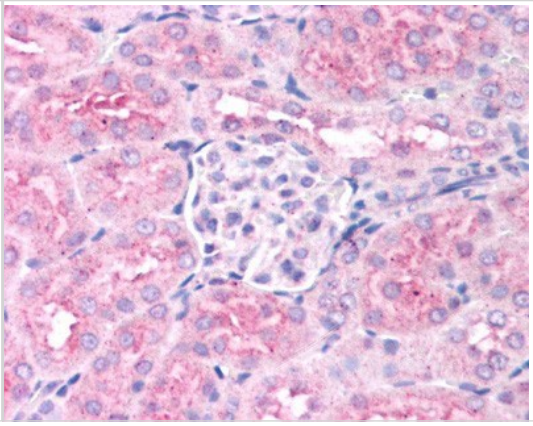
Western Blot: PHD2/HIF Prolyl Hydroxylase 2 Antibody [NB100-2219] - Detection of PHD2 in mouse kidney lysate using NB 100-2219. ECL exposure, 20 seconds.



Immunocytochemistry/Immunofluorescence: PHD2/HIF Prolyl Hydroxylase 2 Antibody [NB100-2219] - PHD2/HIF Prolyl Hydroxlase antibody (1:500) was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



Immunohistochemistry: PHD2/HIF Prolyl Hydroxylase 2 Antibody [NB100-2219] - Staining of renal tubular epithelium in mouse using NB100-2219 at 2.5ug/ml. Mouse kidney cortex, 40X.



Publications

Haag D. Rolle der Siah-Ubiquitinligasen in der Entstehung der pulmonalen Hypertonie und daraus resultierenden rechtsventrikularen Hypertrophie. Einleitung. 2014 (WB, Mouse)

Hesse AR, Levent E, Zieseniss A et al. Lights on for HIF-1a: Genetically Enhanced Mouse Cardiomyocytes for Heart Tissue Imaging. Cell. Physiol. Biochem. 2014 Jul 30 [PMID: 25095893] (WB, Mouse)

Details:
PHD2/HIF Prolyl Hydroxylase 2 antibody used for WB on protein extracts of hypoxia exposed ODD-Luc-CMs/Cardiomyocytes derived from an embryonic stem cell (ESC) lines (ODD-Luc ESCs) that was generated from a Tg ROSA26 ODD-Luc/+ mouse (Figure 2D).

Kim J, Kwak HJ, Cha JY et al. The role of prolyl hydroxylase domain protein (PHD) during rosiglitazone-induced adipocyte differentiation. J Biol Chem 2013 Dec 12 [PMID: 24338020] (WB, Mouse)

An X, Xu G, Yang L et al. Expression of hypoxia-inducible factor-1alpha, vascular endothelial growth factor and prolyl hydroxylase domain protein 2 in cutaneous squamous cell carcinoma and precursor lesions and their relationship with histological stages and clinical features. J Dermatol 2013 Dec 20 [PMID: 24354513] (IHC-P, Human)

Hyvarinen J, Hassinen IE, Sormunen R et al. Hearts of hypoxia-inducible factor prolyl 4-hydroxylase-2 hypomorphic mice show protection against acute ischemia-reperfusion injury. J Biol Chem. 2010 Apr 30 [PMID: 20185832] (WB, Mouse)

Pisarcik S, Maylor J, Lu W et al. Activation of Hypoxia-Inducible Factor-1 in Pulmonary Arterial Smooth Muscle Cells by Endothelin-1. Am J Physiol Lung Cell Mol Physiol 2013 Feb 15 [PMID: 23418090] (WB, Rat)

Kuzmanov A, Wielockx B, Rezaei M et al. Overexpression of factor inhibiting HIF-1 enhances vessel maturation and tumor growth via platelet-derived growth factor-C. International Journal of Cancer 2011 Nov 18 [PMID: 22095574]

Hoelscher M, Silter M, Krull S et al. Cardiomyocyte-specific Prolyl-4-hydroxylase domain 2 knock out protects from acute myocardial ischaemic injury. J Biol Chem. 2011 [PMID: 21270129]

Lehmann S, Stiehl DP, Honer M et al. Longitudinal multimodal in vivo imaging of tumor hypoxia its downstream molecular events. PNAS;106(33):14004-14009. 2009 [PMID: 19666490]

Schodel J, Klanke B, Weidemann A et al. HIF-Prolyl Hydroxylases in the Rat Kidney: Physiologic Expression Patterns Regulation in Acute Kidney Injury. Am J Pathol;174(5):1663-1674. 2009 [PMID: 19349364] (WB, Mouse)

Mikhaylova O, Ignacak ML, Barankiewicz TJ et al. The von Hippel-Lindau tumor suppressor protein and Egl-9-Type proline hydroxylases regulate the large subunit of RNA polymerase II in response to oxidative stress. Mol Cell Biol;28 (8):2701-17. 2008 Apr. [PMID: 18285459] (WB, Human)

Rabie T, Kunze R, Marti HH. Impaired hypoxic response in senescent mouse brain. Int J Dev Neurosci. 2011 Jun 16. [PMID: 21704147]

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.

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.

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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.

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