Product Datasheet

EGLN1/PHD2 Antibody
NB100-137

Unit Size: 0.1 mg

Store at 4C. Do not freeze.

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# NB100-137
EGLN1/PHD2 Antibody

## Product Information

<table>
<thead>
<tr>
<th><strong>Unit Size</strong></th>
<th>0.1 mg</th>
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<tbody>
<tr>
<td><strong>Concentration</strong></td>
<td>1 mg/ml</td>
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<tr>
<td><strong>Storage</strong></td>
<td>Store at 4C. Do not freeze.</td>
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<tr>
<td><strong>Clonality</strong></td>
<td>Polyclonal</td>
</tr>
<tr>
<td><strong>Preservative</strong></td>
<td>0.09% Sodium Azide</td>
</tr>
<tr>
<td><strong>Isotype</strong></td>
<td>IgG</td>
</tr>
<tr>
<td><strong>Purity</strong></td>
<td>Immunogen affinity purified</td>
</tr>
<tr>
<td><strong>Buffer</strong></td>
<td>Tris-Citrate/Phosphate (pH 7.0 - 8.0)</td>
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<tr>
<td><strong>Target Molecular Weight</strong></td>
<td>46 kDa</td>
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## Product Description

<table>
<thead>
<tr>
<th><strong>Host</strong></th>
<th>Rabbit</th>
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<tbody>
<tr>
<td><strong>Gene ID</strong></td>
<td>54583</td>
</tr>
<tr>
<td><strong>Gene Symbol</strong></td>
<td>EGLN1</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td>Human, Mouse, Rat, Primate</td>
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</table>

### Reactivity Notes

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). Primate reactivity reported in scientific literature (PMID: 25974097).

### 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, Knockdown Validated

### Recommended Dilutions

Western Blot 1:500-1:2500, Simple Western 1:500, Flow Cytometry 3.0 mcg/ml, Immunocytochemistry 1:10-1:500, Immunofluorescence 1:50, Immunoprecipitation, Immunohistochemistry-Paraffin 1:10-1:500, Knockdown Validated

### Application Notes

This PHD2 antibody is useful for Flow Cytometry, Immunocytochemistry/Immunofluorescence, Western Blot, and Immunohistochemistry-paraffin embedded sections. In ICC/IF, cytoplasmic 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.
Immunocytochemistry/Immunofluorescence: EGLN1/PHD2 Antibody [NB100-137] - Staining of endogenous PHD2 in U2OS cells. Image from verified customer review.

Immunohistochemistry: EGLN1/PHD2 Antibody [NB100-137] - Staining of Vascular Endothelium 40X in Lung.

Flow Cytometry: EGLN1/PHD2 Antibody [NB100-137] - An intracellular stain was performed on Jurkat cells with EGLN1/PHD2 Antibody 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 expression in human prostate cancer cell lines. Image from verified customer review.

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-PHD2 antibody. Image from verified customer review.

Western Blot: EGLN1/PHD2 Antibody [NB100-137] - Detection of Human PHD2 by Western Blot. Samples: Recombinant epitope-tagged PHD1, PHD2 or PHD3 (10 ng/lane) or whole cell lysate from HeLa cells. Antibody: Affinity purified rabbit anti-PHD2 used at 1 ug/ml. Detection: Chemiluminescence.

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 (http://www.nature.com/articles/cddis2014295), licensed under a CC-BY licence.
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).

Flow Cytometry: EGLN1/PHD2 Antibody [NB100-137] - Detection of PHD2. 1 million Jurkat cells were fixed, permeabilized, and stained with 3.0 µg/ml anti-PHD2 NB100-137 in a 150 ul reaction. Isotype control (black), anti-MLL1 (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.
<table>
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<tr>
<th>Publications</th>
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<tbody>
<tr>
<td>Bo H Role of oxygen on Nuclear factor erythroid-2-like 1 (NFE2L1 or Nrf1) function and stability Thesis (WB, Human)</td>
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<tr>
<td>Miles AL, Burr SP, Grice GL, Nathan JA. The vacuolar-ATPase complex and assembly factors, TMEM199 and CCDC115, control HIF1a prolyl hydroxylation by regulating cellular iron levels. Elife. Mar 15 2017 12:00AM [PMID: 28296633] (ICC/IF, Human)</td>
</tr>
<tr>
<td>More publications at <a href="http://www.novusbio.com/NB100-137">http://www.novusbio.com/NB100-137</a></td>
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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).
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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