

# Product Datasheet

## EGLN3/PHD3 Antibody (EG188e/d5) - BSA Free NBP1-30440

Unit Size: 0.2 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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**NBP1-30440**

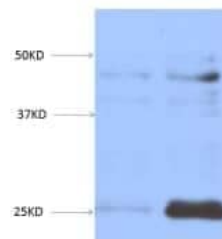
EGLN3/PHD3 Antibody (EG188e/d5) - BSA Free

Product Information	
Unit Size	0.2 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	EG188e/d5
Preservative	0.05% Sodium Azide
Isotype	IgG1
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	27 kDa
Product Description	
Host	Mouse
Gene ID	112399
Gene Symbol	EGLN3
Species	Human, Rat
Immunogen	Partial recombinant human PHD3/HIF Prolyl Hydroxylase 3 (UniProt# Q9H6Z9)
Product Application Details	
Applications	Western Blot, Simple Western, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 10 ug/ml, Simple Western 1:1000, Immunohistochemistry 1:400, Immunohistochemistry-Paraffin 1:400
Application Notes	<p>This HIF Prolyl Hydroxylase 3 antibody is useful for Immunohistochemistry paraffin embedded sections and Western blot, where a band is seen approx. 27 kDa. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.</p> <p>In Simple Western only 10 - 15 uL of the recommended dilution is used per data point.</p> <p>See <a href="#">Simple Western Antibody Database</a> for Simple Western validation: Tested in Hypoxic HeLa lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:1000, apparent MW was 32 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.</p>

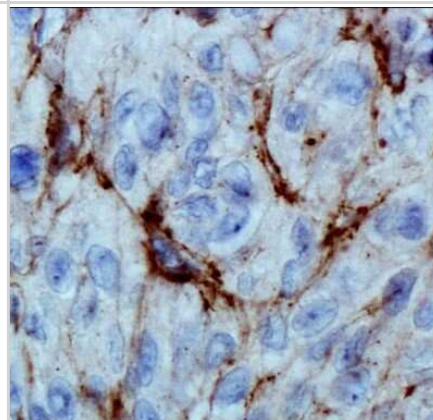


## Images

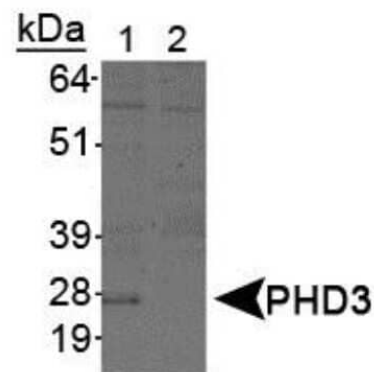
Western Blot: EGLN3/PHD3 Antibody (EG188e/d5) [NBP1-30440] - PHD3 expression in SK-N-BE(2) whole cell lysates. Cells were treated in normoxia (left) and in hypoxia (right). Image from verified customer review.



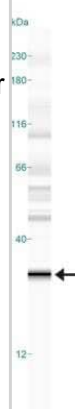
Immunohistochemistry: EGLN3/PHD3 Antibody (EG188e/d5) [NBP1-30440] - Analysis of HIF Prolyl Hydroxylase 3 in human renal cancer using DAB with hematoxylin counterstain.



Western Blot: EGLN3/PHD3 Antibody (EG188e/d5) [NBP1-30440] - Analysis of HIF Prolyl Hydroxylase 3 in (1) Cobalt chloride treated COS-7 nuclear extracts and (2) Untreated COS-7 nuclear extracts.



Simple Western: EGLN3/PHD3 Antibody (EG188e/d5) [NBP1-30440] - Lane view shows a specific band for PHD3/HIF Prolyl Hydroxylase 3 in 0.5 mg/ml of Hypoxic HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



## Publications

- Lock MC, Botting KJ, Allison BJ et al. MitoQ as an antenatal antioxidant treatment improves markers of lung maturation in healthy and hypoxic pregnancy The Journal of physiology 2023-07-19 [PMID: 37467062] (WB, Sheep)
- Pavlakakis D, Kampantais S, Gkagkalidis K et al. Hypoxia-Inducible Factor 2a Expression Is Positively Correlated With Gleason Score in Prostate Cancer Technology in cancer research & treatment 2021-03-23 [PMID: 33752529] (IHC-P, Human)
- Zhang G H, Yu F C et al. Prolyl 4-Hydroxylase Domain Protein 3 Overexpression Improved Obstructive Sleep Apnea-Induced Cardiac Perivascular Fibrosis Partially by Suppressing Endothelial-to-Mesenchymal Transition. J Am Heart Assoc 2017-10-19 [PMID: 29051216] (WB, Human)
- Capitanio D, Moriggi M et al. Comparative proteomic analyses of Duchenne muscular dystrophy and Becker muscular dystrophy muscles: changes contributing to preserve muscle function in Becker muscular dystrophy patients. J Cachexia Sarcopenia Muscle 2020-01-04 [PMID: 31991054] (WB, Human)
- Bur H, Haapasaari K M et al. Strong Prolyl Hydroxylase Domain 1 Expression Predicts Poor Outcome in Radiotherapy-treated Patients with Classical Hodgkin's Lymphoma. Anticancer Res 2018-01-01 [PMID: 29277791] (IF/IHC, Human)
- Mysore VS, Szablowski J, Dervan PB, Frost PJ. A DNA-binding Molecule Targeting the Adaptive Hypoxic Response in Multiple Myeloma Has Potent Antitumor Activity. Mol Cancer Res. [PMID: 26801054] (WB, Human)
- Fujita N, Gogate SS, Chiba K et al. Prolyl Hydroxylase 3 (PHD3) Modulates Catabolic Effects of Tumor Necrosis Factor-alpha (TNF-alpha) on Cells of the Nucleus Pulposus through Co-activation of Nuclear Factor kappaB (NF-kappaB)/p65 Signaling J Biol Chem 2012-11-16 [PMID: 22948157] (WB, Rat)
- Soilleux EJ, Turley H, Tian YM et al. Use of novel monoclonal antibodies to determine the expression and distribution of the hypoxia regulatory factors PHD-1, PHD-2, PHD-3 and FIH in normal and neoplastic human tissues. Histopathology. 2005-12-01 [PMID: 16324198] (IHC-P, Human)
- Stolze IP, Tian YM, Appelhoff RJ et al. Genetic analysis of the role of the asparaginyl hydroxylase factor inhibiting hypoxia-inducible factor (FIH) in regulating hypoxia-inducible factor (HIF) transcriptional target genes [corrected]. J Biol Chem. 2004-10-01 [PMID: 15302861]
- Appelhoff RJ et al. Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. J Biol Chem 279: 38458-38465. 2004-01-01 [PMID: 15247232] (WB, Human)



## Procedures

### Western Blot protocol specific for PHD3 Antibody (NBP1-30328)

#### Western Blot Protocol

1. Perform SDS-PAGE (4-12% MOPS) 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 dH<sub>2</sub>O 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% NFDM + 1% BSA in TBS + Tween, 1 hour at RT.
6. Rinse the membrane in dH<sub>2</sub>O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the mouse anti-PHD3 primary antibody (NBP1-30328) in blocking buffer and incubate 1 hour at room temperature.
8. Rinse the membrane in dH<sub>2</sub>O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted mouse-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
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 manufacturers instructions (Pierce 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.

### Immunohistochemistry-Paraffin protocol for EGLN3/PHD3 Antibody (NBP1-30440)

#### Immunohistochemistry-Paraffin Embedded Sections

##### Antigen Unmasking:

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

##### Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.





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### **Products Related to NBP1-30440**

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HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-97005-0.5mg	Mouse IgG1 Isotype Control (MG1)
NBP1-30440H	EGLN3/PHD3 Antibody (EG188e/d5) [HRP]

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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.

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