abcam

# ab108980

# Anti-PHD1/prolyl hydroxylase antibody [EPR2745]

Recombinant | RabMAb

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA |
| **Form** | Liquid |
| **Clonality** | Monoclonal |
| **Immunogen** | The exact immunogen used to generate this antibody is proprietary information. |
| **Clone number** | EPR2745 |
| **Purification technique** | Affinity purification Protein A |
| **Concentration** | 0.408 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

## IHC-P

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/100 - 1/250 |
| **Notes** | Perform antigen retrieval<br>Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. |

### Predicted

| | |
|---|---|
| **Species** | Mouse, Rat |
| **Dilution info** | - |
| **Notes** | - |

## IP

### Not recommended

| | |
|---|---|
| **Species** | Human, Mouse, Rat |
| **Dilution info** | - |
| **Notes** | - |

## WB

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/1000 - 1/10000 |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Mouse, Rat |
| **Dilution info** | - |
| **Notes** | - |

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/100 - 1/250 |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Mouse, Rat |
| **Dilution info** | - |
| **Notes** | - |

## Flow Cyt (Intra)

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/10 - 1/100 |
| **Notes** | ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. |

### Predicted

| | |
|---|---|
| **Species** | Mouse, Rat |
| **Dilution info** | - |
| **Notes** | - |

## Target data

See full target information EGLN2

**Function**

Prolyl hydroxylase that mediates hydroxylation of proline residues in target proteins, such as ATF4, IKBKB, CEP192 and HIF1A (PubMed:11595184, PubMed:12039559, PubMed:15925519, PubMed:16509823, PubMed:17114296, PubMed:23932902). Target proteins are preferentially recognized via a LXXLAP motif (PubMed:11595184, PubMed:12039559, PubMed:15925519). Cellular oxygen sensor that catalyzes, under normoxic conditions, the post-translational formation of 4-hydroxyproline in hypoxia-inducible factor (HIF) alpha proteins (PubMed:11595184, PubMed:12039559, PubMed:12181324, PubMed:15925519, PubMed:19339211). Hydroxylates a specific proline found in each of the oxygen-dependent degradation (ODD) domains (N-terminal, NODD, and C-terminal, CODD) of HIF1A (PubMed:11595184, PubMed:12039559, PubMed:12181324, PubMed:15925519). Also hydroxylates HIF2A (PubMed:11595184, PubMed:12039559, PubMed:15925519). Has a preference for the CODD site for both HIF1A and HIF2A (PubMed:11595184, PubMed:12039559, PubMed:15925519). Hydroxylated HIFs are then targeted for proteasomal degradation via the von Hippel-Lindau ubiquitination complex (PubMed:11595184, PubMed:12039559, PubMed:15925519). Under hypoxic conditions, the hydroxylation reaction is attenuated allowing HIFs to escape degradation resulting in their translocation to the nucleus, heterodimerization with HIF1B, and increased expression of hypoxy-

inducible genes (PubMed:11595184, PubMed:12039559, PubMed:15925519). EGLN2 is involved in regulating hypoxia tolerance and apoptosis in cardiac and skeletal muscle (PubMed:11595184, PubMed:12039559, PubMed:15925519). Also regulates susceptibility to normoxic oxidative neuronal death (PubMed:11595184, PubMed:12039559, PubMed:15925519). Links oxygen sensing to cell cycle and primary cilia formation by hydroxylating the critical centrosome component CEP192 which promotes its ubiquitination and subsequent proteasomal degradation (PubMed:23932902). Hydroxylates IKBKB, mediating NF-kappa-B activation in hypoxic conditions (PubMed:17114296). Also mediates hydroxylation of ATF4, leading to decreased protein stability of ATF4 (By similarity).

## Storage

| | |
|---|---|
| **Shipped at conditions** | Conditional Ambient |
| **Appropriate short-term storage duration** | 1-2 weeks |
| **Appropriate short-term storage conditions** | +4°C |
| **Appropriate long-term storage conditions** | -20°C |
| **Aliquoting information** | Upon delivery aliquot |
| **Storage information** | Avoid freeze / thaw cycle |

## Notes

**Patented technology**
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

**What are the advantages of a recombinant monoclonal antibody?**
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

## Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

| | |
|---|---|
| **Activity summary** | Prolyl hydroxylase 1 (PHD1) also known as EGLN2 is an enzyme that hydroxylates specific proline residues within target proteins. This process of prolyl hydroxylation is critical for regulating protein stability. PHD1 shares structural similarities with other members of the 2-oxoglutarate-dependent dioxygenase family and includes a conserved iron-binding motif essential for its enzymatic activity. PHD1 has a molecular mass of approximately 46 kDa and is expressed in a variety of tissues with notable presence in skeletal muscle heart and the liver. |
| **Biological function summary** | PHD1 modulates the stability of the hypoxia-inducible factor (HIF) proteins which are key regulators of oxygen homeostasis in the cell. Under normoxic conditions PHD1 hydroxylates specific proline residues on HIF-alpha marking it for degradation via the ubiquitin-proteasome pathway. This enzyme does not function as part of a larger protein complex but it plays a pivotal role in determining the cellular response to oxygen levels. Additionally PHD1 expression affects the metabolic adaptation processes and energy expenditure in cells. |
| **Pathways** | PHD1 plays a role in the cellular response to hypoxia and is integral to the HIF signaling pathway. It interacts directly with HIF-alpha subunits mediating their degradation under normal oxygen conditions to ensure HIF activity remains inhibited. Additionally PHD1 is indirectly involved in modulating the angiogenesis pathway as it influences the availability of HIF-related transcription factors which promote transcription of vascular endothelial growth factor (VEGF) under low oxygen conditions. The interplay between PHD1 PHD2 and PHD3 ensures a fine-tuned regulation of the HIF pathway based on oxygen availability. |
| **Associated diseases and disorders** | PHD1 has links to cancer progression and ischemic conditions. The enzyme's activity is often altered in response to the aberrant hypoxic signaling found within tumors impacting cellular proliferation and survival. In ischemic conditions reduced PHD1 activity leads to stabilization of HIF proteins and adaptation responses aimed at tissue survival. Mutations or dysregulation in PHD1 expression have been observed in metabolic syndromes suggesting potential therapeutic targets. Through its control over hypoxia-related proteins PHD1 also interacts with von Hippel-Lindau (VHL) protein which is part of the E3 ubiquitin ligase complex important for HIF-alpha degradation. |

## Product promise

**Tested**

We have tested this species and application combination and it works. It is covered by our product promise.

**Expected**

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

**Predicted**

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

**Not recommended**

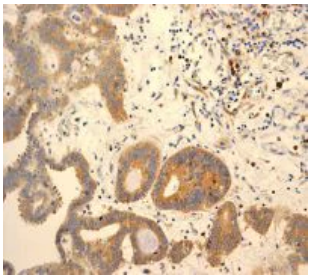
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

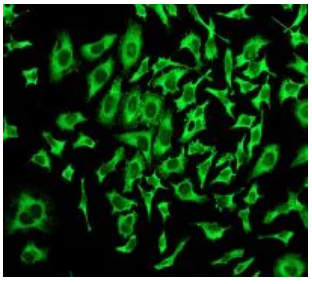
## 7 product images



### Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PHD1/prolyl hydroxylase antibody [EPR2745] (ab108980)

PHD1/prolyl hydroxylase Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PHD1/prolyl hydroxylase antibody

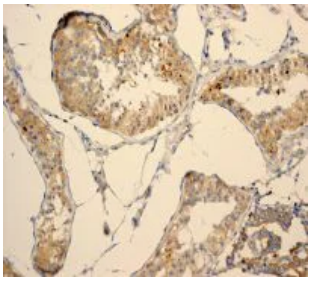
Immunohistochemical analysis of paraffin-embedded Human ovarian adenocarcinoma tissue using ab108980
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



### Immunocytochemistry/ Immunofluorescence - Anti-PHD1/prolyl hydroxylase antibody [EPR2745] (ab108980)

PHD1/prolyl hydroxylase Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-PHD1/prolyl hydroxylase antibody

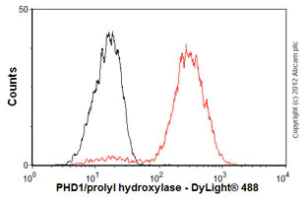
Immunofluorescent staining of HeLa cells using ab108980



### Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PHD1/prolyl hydroxylase antibody [EPR2745] (ab108980)

PHD1/prolyl hydroxylase Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PHD1/prolyl hydroxylase antibody

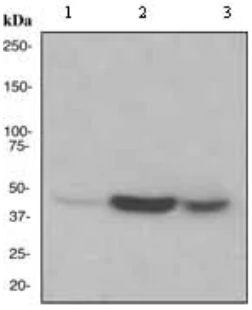
Immunohistochemical analysis of paraffin-embedded Human testis tissue using ab108980
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.




## Flow Cytometry (Intracellular) - Anti-PHD1/prolyl hydroxylase antibody [EPR2745] (ab108980)

PHD1/prolyl hydroxylase Flow Cytometry (Intracellular) staining using rabbit Anti-PHD1/prolyl hydroxylase antibody

Overlay histogram showing HeLa cells stained with ab108980 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab108980 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monclonal) (1µg/1x$10^6$ cells) used under the same conditions. Acquisition of >5000 events was performed.




## Western blot - Anti-PHD1/prolyl hydroxylase antibody [EPR2745] (ab108980)

All lanes:
Western blot - Anti-PHD1/prolyl hydroxylase antibody [EPR2745] (ab108980) at 1/1000 dilution

Lane 1:
A549 cell lysate at 10 µg

Lane 2:
HeLa cell lysate at 10 µg

Lane 3:
MCF-7 cell lysate at 10 µg

Secondary

All lanes:
Standard HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 44 kDa

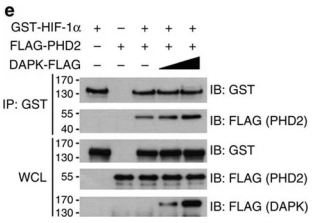



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## Western blot - Anti-PHD1/prolyl hydroxylase antibody [EPR2745] (ab108980)

PHD1/prolyl hydroxylase Western blot staining using rabbit Anti-PHD1/prolyl hydroxylase antibody

Western Blotting using Anti-PHD1/prolyl hydroxylase antibody [EPR2745], ab108980. Publication image from Hsu, T. S. et al., 2016, Nat Commun, 27312851. Legend direct from paper.

DAPK interacts with PHD2 and increases PHD2-HIF-1α association.(a) Interaction of DAPK with PHD2. Jurkat cells were transfected with FLAG-PHD2 and DAPK-Myc. The whole-cell lysates (WCL) were immunoprecipitated with anti-Myc, and the contents of FLAG-PHD2 and DAPK-Myc in the precipitates and WCL were determined by anti-FLAG and anti-Myc. (b) Co-localization of DAPK with PHD2 in Th17 cells. WT naive T cells were differentiated into Th17 for 2 days, and cells were fixed. Fixed cells were stained with anti-PHD2, anti-DAPK and DAPI, and analysed by confocal microscopy. Scale bar, 2.5 µm. (c) Co-localization of HIF-1α with PHD2 in Th17 cells. Th17 cells were stained with anti-PHD2, anti-HIF-1α and DAPI, and analysed by confocal microscopy. Scale bar, 2.5 µm. (d) DAPK interacts with the N-terminal domain of PHD2. HEK293T cells were transfected with DAPK-Myc, and the N-terminal (aa 1–180) or C-terminal (aa 181–426) domain of FLAG-PHD2 as indicated. Cell lysates were immunoprecipitated with anti-Myc, and the contents of

PHD2 and DAPK were determined. (e) DAPK increases the association of PHD2 with HIF-1α in vitro. Recombinant GST-HIF-1α protein was incubated with FLAG-PHD2 in the absence or presence of recombinant DAPK-FLAG proteins in PBS at 4 °C for 4 h. The GST-HIF-1α complex was pulled down by anti-GST, and the contents of FLAG-PHD2, GST-HIF-1α and DAPK-FLAG in the precipitates and incubation mixtures were determined. Data (a–e) are representative of two independent experiments.

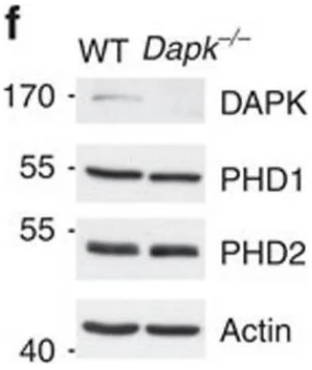



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PHD1/prolyl hydroxylase Western blot staining using rabbit Anti-PHD1/prolyl hydroxylase antibody

Western Blotting using Anti-PHD1/prolyl hydroxylase antibody [EPR2745], ab108980. Publication image from Hsu, T. S. et al., 2016, Nat Commun, 27312851. Legend direct from paper.

DAPK promotes PHD2-dependent HIF-1α degradation.(a) Deletion of the oxygen-dependent degradation (ODD) region confers resistance of HIF-1α to DAPK-induced degradation. HEK293T cells were transfected with DAPK-FLAG and HIF-1α[δODD] and the levels of HIF-1α were determined 48 h after transfection. (b) PHD inhibitor prevents DAPK-induced HIF-1α degradation in normal T cells. Naive T cells were activated for 48 h and transduced with vector or DAPK-FLAG-IRES-GFP. GFP+ T cells were sorted 48 h later, switched to hypoxic incubation (1% O2) in the absence or presence of DFX (10 μM) for another 24 h, and the levels of DAPK-FLAG and HIF-1α were determined. (c) DAPK deficiency decreases proline hydroxylation of HIF-1α in normal T cells. Naive WT and Dapk−/− T cells were activated with anti-CD3/CD28 for 48 h in normoxic conditions, followed by treatment with MG132 (10 μM). The extents of Pro564 hydroxylation on HIF-1α were determined at the indicated time points. (d) DAPK enhances proline hydroxylation of HIF-1α. YFP control and DAPK-FLAG-expressing Jurkat cells were treated with MG132 (5 μM) in normoxic conditions. The extents of Pro564 hydroxylation on HIF-1α were determined at the indicated time points. (e) Mutation of proline hydroxylation sites confers resistance of HIF-1α to DAPK-induced degradation in T cells. Jurkat cells were transfected with DAPK-FLAG, HIF-1α-WT or HIF-1α[P402A/P564A] (HIF-1α-2PA), as indicated. Twenty-four hours after transfection, cells were incubated under hypoxic conditions (1% O2) for 6 h, then switched back to normoxia for 10 min, and the levels of HIF-1α were determined. (f) DAPK deficiency does not affect the expression of PHD1 and PHD2. WT and Dapk−/− naive CD4 T cells were allowed to differentiate into Th17 cells for 3 days. Cells were harvested and the expression of PHD1 and PHD2 was examined. (g) PHD2-knockdown prevents DAPK-induced HIF-1α degradation in T cells. Jurkat T cells were transfected with DAPK-FLAG, siRNA control (siCtrl), or siRNA for PHD2 (siPHD2). Twenty-four hours later, cells were switched to hypoxic conditions for 6 h, and the levels of HIF-1α, PHD2 and HSP90 were determined. Data are representative of three (a,d) or two (b,c,e–g) independent experiments.

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