

DESCRIPTION

Species Reactivity	Human/Rat
Specificity	Detects human EGLN/PHD2 in direct ELISAs. Detects human and rat EGLN/PHD2 in Western blots. In direct ELISAs, no cross-reactivity with human PHD1 and PHD3 is observed.
Source	Recombinant Monoclonal Rabbit IgG Clone # 2445B
Purification	Protein A or G purified from cell culture supernatant
Immunogen	<i>S. frugiperda</i> insect ovarian cell line Sf 21-derived recombinant human EGLN1/PHD2 Ala2-Phe426 Accession # Q9GZT9
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

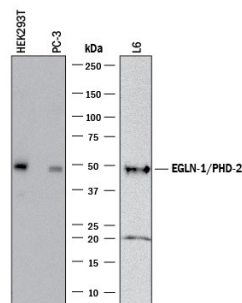
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. [General Protocols](#) are available in the Technical Information section on our website.

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Flow Cytometry	0.25 µg/10 ⁶ cells	See Below
Immunocytochemistry	3-25 µg/mL	See Below
Immunohistochemistry	3-25 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

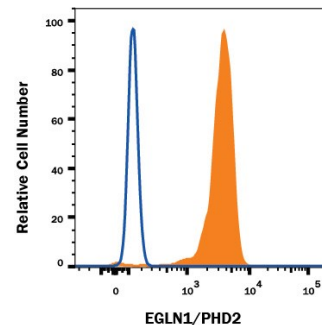
DATA

Western Blot



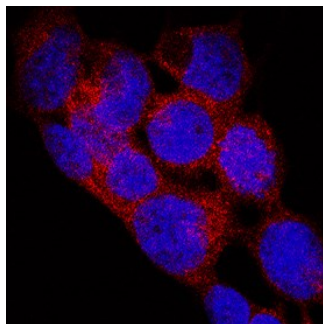
Detection of Human and Rat EGLN1/PHD2 by Western Blot. Western blot shows lysates of HEK293T human embryonic kidney cell line, PC-3 human prostate cancer cell line, and L6 rat myoblast cell line. PVDF membrane was probed with 1 µg/mL of Rabbit Anti-Human/Rat EGLN1/PHD2 Monoclonal Antibody (Catalog # MAB7680) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for EGLN1/PHD2 at approximately 49 kDa (as indicated). This experiment was conducted under reducing conditions and using [Immunoblot Buffer Group 1](#).

Flow Cytometry



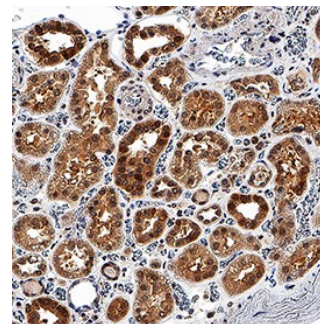
Detection of EGLN1/PHD2 in Human Jurkat cell line by Flow Cytometry. Human Jurkat T Cell Leukemia Cell Line was stained with Rabbit Anti-Human/Rat EGLN1/PHD2 Monoclonal Antibody (Catalog # MAB7680, filled histogram) or Rabbit IgG Isotype Control Antibody (Catalog # MAB1050, open histogram) followed by Phycoerythrin-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0110). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (Catalog # FC012). View our protocol for [Staining Membrane-associated Proteins](#).

Immunocytochemistry



EGLN1/PHD2 in WM-115 Human Cell Lines. EGLN1/PHD2 was detected in immersion fixed WM-115 human malignant melanoma cell line using Rabbit Anti-Human/Rat EGLN1/PHD2 Monoclonal Antibody (Catalog # MAB7680) at 3 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to cell cytoplasm and nuclei. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunohistochemistry



EGLN1/PHD2 in Human Kidney. EGLN1/PHD2 was detected in immersion fixed paraffin-embedded sections of human kidney using Rabbit Anti-Human/Rat EGLN1/PHD2 Monoclonal Antibody (Catalog # MAB7680) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cell cytoplasm and nuclei. View our protocol for [IHC Staining with VisUCyte HRP Polymer Detection Reagents](#).

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none">• 12 months from date of receipt, -20 to -70 °C as supplied.• 1 month, 2 to 8 °C under sterile conditions after reconstitution.• 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

PHD2 (Prolyl Hydroxylase Domain-containing protein 2; also HPH2, EGLN1 and HIF-PH2) is a 45-47 kDa dioxygenase member of the PH family of enzymes. It is ubiquitously expressed, and serves to regulate the availability of the oxygen-sensitive HIF transcription factor. Active HIF1 α is a heterodimer of α - and β -subunits and when intact, promotes VEGF and EPO production. The β -subunit is constitutively expressed, while α -subunit levels are regulated by intracellular oxygen concentration. At normoxic levels, the α -subunit is hydroxylated on Pro by one of three PHDs, inducing its ubiquitination/degradation. The hydroxylation event requires oxygen, and thus PH activity (particularly PHD2) is a measure of a cell's oxygen concentration. Human PHD2 is 426 amino acids (aa) in length. It contains an NES (aa 6-20), a Zn-finger region (aa 21-58), and a catalytic domain (aa 291-392). There are five nitrosylated cysteines plus one acetylated alanine. Two isoform variants are known, one that shows a deletion of aa 338-359, and another that contains a 17 aa substitution for aa 58-175. Over aa 157-426, human PHD2 shares 93% aa sequence identity with mouse PHD2.