ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

HIF-Prolyl hydroxylase 2 (PHD2/EGLN1) Antibodies

Cat. PHD21-P	Human PHD2 control/blocking peptide # 1	**SIZE**: 100 ug
Cat. PHD21-A	**Rabbit** Anti- Human PHD2 IgG # 1 (aff pure)	**SIZE**: 100 ug

Oxygen is absolutely critical for the survival of mammalian cells. Hypoxia induced factor (HIF) is a transcriptional complex that plays a central role in mammalian oxygen homeostasis. There are 3-types of alpha subunits (HIF1-3alpha) and one HIF-beta subunits. However, the three HIF-alpha subunits are regulated by oxygen in a similar fashion, i.e. by regulated stabilization of the alpha-subunits. Under normal conditions, HIF-alpha Prolines are hydroxylated at Pro-402 and Pro-564. This allows binding of von Hippel-Lindau (VHL), the substrate recognition component of the E3 ubiquinated ligase complex, subsequent ubiquitination and degradation of HIF-alpha by the proteasome. Under hypoxic conditions, hydroxylation of HIF-alpha is inhibited and this prevents HIF-alpha degradation. The enzymes responsible for HIF-hydroxylation are known as HIF-prolyl hydroxylases (PHD1-3 or HPH1, HPH2, and HPH3). The three PHDs have been identified to hydroxylate the motif, LXXLAP* with *P being the hydroxyproline.

PHD2 (human 426 aa, chromosome 1q42-q43, also known as Egl nine homolog 1, EGLN1, (Hypoxia-inducible factor prolyl hydroxylase 2) (HIF-prolyl hydroxylase 2) (HIF-PH2) (HPH-2) (Prolyl hydroxylase domain-containing protein 2) PHD2 (SM-20) (PNAS-118 / PNAS-137) is widely expressed. Alternatively spliced isoforms 2 is missing 337-358 aa. It is activated by hypoxia in some cells and tissues.

FUNCTION: Catalyzes the post-translational formation of 4-hydroxyproline in hypoxia-inducible factor (HIF) alpha proteins. Hydroxylates HIF-1 alpha at 'Pro-402' and 'Pro-564', and HIF-2 alpha. Functions as a cellular oxygen sensor and, under normoxic conditions, targets HIF through the hydroxylation for proteasomal degradation via the von Hippel-Lindau ubiquitination complex.
SIMILARITY: Contains 1 MYND-type zinc finger.
Protein name Egl nine homolog 1
Synonyms EC 1.14.11.-, Hypoxia-inducible factor prolyl hydroxylase 2, HIF-prolyl hydroxylase 2, HIF-PH2, HPH-2
Prolyl hydroxylase domain-containing protein 2
PHD2, SM-20
Gene name Name: EGLN1; Synonyms: C1orf12
ORFNames: PNAS-118, PNAS-137

Source of Antigen and Antibodies

Antigen	16-aa peptide of Human PHD2/EGLN1 (1) (protein accession #Q9GZT9 , refs 1) ; **Designated (PHD21-P or control peptide)** conjugated to KLH; **.**Epitope location ~C-terminal
Ab Host/type	Rabbit, Polyclonal IgG, (Cat # PHD21-A) purified over the antigen column
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available).
-ve control	# 20009-1, Rabbit (non-immune) IgG, purified, suitable for ELISA, Western, IHC as –ve control

Form & Storage of Antibodies/Peptide Control

Affinity pure IgG
100 ug/100ul solution lyophilized powder
Supplied in **Buffer**: PBS+0.1% BSA
Reconstitute powder in PBS at 1mg/ml

Control/blocking peptide
100 ug/100 ul solution lyophilized powder
Supplied in Buffer: PBS pH 7.5,
Reconstitute powder in PBS at 1 mg/ml.

Storage

Short-term: unopened, undiluted liquid vials at -20^{O}C and powder at 4^{o}C or -20^{o}C..

Long-term: at –20^{0}C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20^{o}C or below.
Shipping: 4^{o}C for solutions and room temp for powder

Recommended Usage

Western Blotting 1-5 ug/ml for affinity pure using Chemiluminescence technique.

ELISA (1:10K-1:100K; using 50-100 ng of control peptide/well).

Histochemistry & Immunofluorescence: Not tested.

Specificity & Cross-reactivity

Human PHD21-P sequence is 87% conserved in chicken and only 68% in mouse and rat PHD2. No significant sequence homology exist with other PHDs. Antibody crossreactivity in various species has not been studied. Control peptide, because of its low mol. Wt (<3 kDa), is not suitable for Western. It should be used for ELISA or antibody blocking experiments (use 5-10 ug control peptide per 1 ug of aff pure IgG or 1 ul antiserum) to confirm antibody specificity

General References: Dupuy D et al (2000) Genomincs 69, 348-354; Taylor MS et al (2001) Gene 275, 125-132; Semenza GL et al (2001) Cell, 107, 1-3; Epstein ACR et al (2001) Cell 107, 43-54; Ivan M et al (2002) PNAS 99, 13459-13464; Cioffi CL et al BBRC 303, 947-953

*This product is for in vitro research use only.

Related material available from ADI

Antibodies to PHD1-3, HIF1-3, and other apoptosis proteins

Western Blot recycling kit (Use the same blot to probe with multiple antibodies NBC1-3)

ReadyBlot brain and Kidney Explorer (study distribution of proteins in pre-made protein

PHD21-A-P 70912J

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