ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

HIF-Prolyl hydroxylase 3 (PHD3/EGLN3) Antibodies

Cat. PHD31-P	Human PHD3 control/blocking peptide # 1	**SIZE**: 100 ug
Cat. PHD31-A	**Rabbit** Anti- Human PHD3 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.

PHD3 (human 333-aa, chromosome 14; also known as EGL9, C. ELEGANS, HOMOLOG OF, 3, EGLN3, PROLYL HYDROXYLASE DOMAIN-CONTAINING PROTEIN 3;PHD3, HIF-PROLYL HYDROXYLASE 3; HIFPH3) Widely expressed at low levels. Expressed at higher levels in heart (cardiac myocytes, aortic endothelial cells and coronary artery smooth muscle) and placenta. It is activated under hypoxia.

FUNCTION: Catalyzes the post-translational formation of 4-hydroxyproline in hypoxia-inducible factor (HIF) alpha proteins. Hydroxylates HIF-1 alpha at '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. May play a role in cell growth regulation in muscle cells and in apoptosis in neuronal tissue. Promotes cell death through a caspase-dependent mechanism (By similarity).
SUBCELLULAR LOCATION: Cytoplasm. Nucleus.
SIMILARITY: Contains 1 PKHD (prolyl/lysyl hydroxylase) domain.
Protein name Egl nine homolog 3
Synonyms EC 1.14.11.-, Hypoxia-inducible factor prolyl hydroxylase 3, HIF-prolyl hydroxylase 3, HIF-PH3, HPH-1
Prolyl hydroxylase domain-containing protein 3, PHD3
Gene name Name: EGLN3

Source of Antigen and Antibodies

Antigen	16-aa peptide of Human PHD3/EGLN3 (1) (protein accession #Q9H6Z9 , refs 1) ; **Designated (PHD31-P or control peptide).**conjugated to KLH; Epitope location ~C-terminal
Ab Host/type	Rabbit, Polyclonal Aff pure IgG, (Cat # PHD31-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 PHD31-P sequence is 87% conserved in mouse and rat PHD3. 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: Strausberg RL et al (2002) PNAS 99, 16899-16903; Taylor MS et al (2001) Gene 275, 125-132; Epstein AC et al (2001) Cell 107, 43-54; Semenza GL et al (2001) Cell 107, 1-3; Bruick RK et al (2001) Science 294, 1337-1340; Oehme F et al (2002) BBRC 296, 343-349;

*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

PHD31-A-P 70912J

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