ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

HIF-Prolyl hydroxylase 4 (PH-4) Antibodies

Cat. PH42-P Human PH-4/EGLX control/blocking peptide # 1 **SIZE**: 100 ug

Cat. PH42-A **Rabbit** Anti- PH-4 3 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.

A novel putative proline hydroxylase, PH-4, with an N-terminal EF-hand motif and a C-terminal catalytic domain with 1 transmembrane domain has been identified in endoplasmic reticulum. PH-4 showed approx. 10-15% homology with the collagen prolyl 4-hydroxylases, PH-alpha I, and PH-alpha II. Human PH-4 (502-aa, chromosome 3pp21.3) is highly expressed in most tissues. Like PHD1-3, PH-4 suppressed the HIF transactivation activity, dependent on the consensus oxygen-dependent degradation domain (ODDD) praline residues. Ph-4 levels correlated with the cellular HIF concentration. Therefore, PH-4 may also be involved in cellular oxygen sensing mechanism.

FUNCTION: May catalyze the post-translational formation of 4-hydroxyproline in hypoxia-inducible factor (HIF) alpha proteins. May function as a cellular oxygen sensor and, under normoxic conditions, may target HIF through the hydroxylation for proteasomal degradation via the von Hippel-Lindau ubiquitination complex.
SUBCELLULAR LOCATION: Endoplasmic reticulum membrane; Peripheral membrane protein.
SIMILARITY: Contains 2 EF-hand domains.
Protein name Putative HIF-prolyl hydroxylase PH-4
Synonyms EC 1.14.11.-
Hypoxia-inducible factor prolyl 4-hydroxylase
Gene name Name: PH4

Source of Antigen and Antibodies

Antigen	17-aa peptide of Human PH-4/EGLX (1) (protein accession #Q9NXG6 , refs 1) ; Designated **(PH42-P or control peptide).** conjugated to KLH; Epitope location ~ C-terminus
Ab Host/type	Rabbit, Polyclonal IgG, purified over antigen-agarose (Cat # PH42-A) supplied in PBS+0.1% BSA+0.05% azide
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 PH42-P is 94% conserved in mouse PH-4. No significant homology exist with PHD1-3. 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: Oehme F et al (2002) BBRC 296, 343-349; Strausberg RL et al (2002) PNAS 99, 16899-16903; Ota T et al (2004) Nature Genet. 36, 40-45

*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)

PH42-A-P 70911J

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