NEUROMICS

HIF Prolyl Hydroxylase 2 Data Sheet

Catalog Number:	MO25046	**Host:**	Mouse
Product Type:	Protein G purified IgG_1. Clone: EGLN1	**Species Reactivity:**	Human
Immunogen Sequence:	A peptide within residues 1-50 of the human protein. [Swiss-Prot# Q9GZT9]	**Format:**	Liquid with Tris-glycine, 150mM NaCl pH 7.5. Concentration 1 mg/ml.

Applications: Immunohistochemistry 0.5-2.0 ug/ml (Paraffin-embedded)
Western blot: 0.5-2.0 ug/ml

*Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.

Storage: Store frozen. Aliquot as undiluted antisera and immediately place at -20°C. Antisera may have become trapped in top of vial during shipping. Centrifugation of vial is recommended before opening. Stable for at least 6 months at -20°C. Repeated freeze/thaw cycles compromise the integrity of the antiserum.

Publications:

Appelhoff RJ et al. Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. *J Bil Chem. 279: 38458-38465 (2004)*
Stolze IP et al. Genetic analysis of the role of the asparaginyl hydroxylase factor inhibiting hypoxia-inducible factor (HIF) in regulating HIF transcriptional target genes. *J Bio Chem. 279:42719-42725 (2004)*
Soilleux EJ et al. Use of novel monoclonal antibodies to determine the expression and distribution of the hypoxia regulatory factors PHD-1, PHD-2, PHD-3 and FIH in normal and neoplastic human tissues. *Histopathology 47: 602-610 (2005)*

Application Notes

This antibody is designed for Western blot analysis, where a band is seen at ~46 kDa. This antibody also works for immunohistochemistry in Paraffin embedded tissue,

Western Blot:
1. Perform SDS-PAGE (4-12% MOPS) on samples to be analyzed, loading 30 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH2O and then stain the blot using Ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk + 1% BSA in TBS, 1 hour at room temperature.
6. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-LPIN1 primary antibody (NB 110-57150) in blocking buffer and incubate 2 hours at room temperature.

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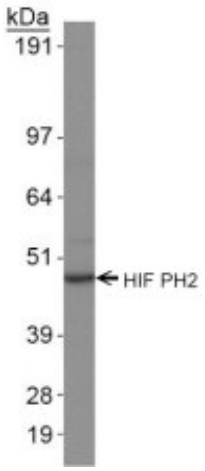
8. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers' instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers' instructions (Pierce, ECL).

Note: Tween-20 can be added to the blocking or antibody diultion buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

Description/Data:
HIF prolyl hydroxylase 2 is a prolyl hydroxylase that modifies HIF-alpha. Classic prolyl hydroxylases are found in the endoplasmic reticulum and modify collagen, whereas HIF is an intracellular protein and the HPH sites do not resemble those modifying collagen. HIF is a transcriptional complex that plays a critical role in oxygen homeostasis. HPH is an essential component of the pathway through which cells sense oxygen. In the presence of oxygen, HPHs convert specific prolyl residues in HIF-alpha to hydroxyproline, leading to HIF-alpha destruction. Low oxygen levels, sensed at the cellular level, cause the HIF conversion to be reduced so that HIF is stable and there is increased angiogenesis. HPH-2, specifically, catalyzes the posttranslational formation of 4-hydroxyproline in HIF alpha proteins. It hydroxylates HIF-1 alpha at Pro(402) and Pro(564), and HIF-2 alpha. It targets HIF through the hydroxylation for proteasomal degradation via the von Hippel-Lindau ubiquitylation complex.

Image: Western blot analysis in HeLa whole cell extracts using HIF Prolyl Hydroxylase 2 Antibody.



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