Recombinant PHD3 (EGLN3) protein



 Catalog No: 81033, 81733
 Quantity: 50, 1000 μg

 Lot No: 24417001
 Concentration: 0.25 μg/μl

Expressed In: E. coli Source: Human

Buffer Contents: Recombinant PHD3 (EGLN3) protein is supplied in 25 mM Tris-HCl pH 8.0, 300 mM NaCl, 10% glycerol and 0.5 mM TCEP.

Background: Prolyl Hydroxylase Domain-Containing Protein 3 (PHD3) or Egl-9 Family Hypoxia Inducible Factor 3 (EGLN3), also known as PHIF-PH3 or HPH-3, is a prolyl hydroxylase. PHD3 is a cellular oxygen sensor that catalyzes the post-translational formation of 4-hydroxyproline in hypoxia-inducible factor (HIF) alpha proteins under normoxic conditions. It can hydroxylate a specific proline found in each of the oxygen-dependent degradation (ODD) domains (N-terminal, NODD, and C-terminal, CODD) of HIF1A, also HIF2A. PHD3 has a preference for the CODD site for both HIF1A and HIF2A. Hydroxylation on the NODD site by PHD3 appears to require prior hydroxylation on the CODD site, and then hydroxylated HIFs are targeted for proteasomal degradation via the von Hippel-Lindau ubiquitination complex. Under hypoxic conditions, the hydroxylation reaction is attenuated allowing HIFs to escape degradation resulting in their translocation to the nucleus, heterodimerization with HIF1B, and increased expression of hypoxy-inducible genes. PHD3 can also hydroxylate PKM in hypoxia, limiting glycolysis, while under normoxia, hydroxylate and regulates the stability of ADRB2. In cardiomyocytes, it inhibits the anti-apoptotic effect of BCL2 by disrupting the BAX-BCL2 complex. In neurons, it has a NGF-induced proapoptotic effect, probably through regulating CASP3 activity. PHD3 is essential for hypoxic regulation of neutrophilic inflammation. Beside, it plays a crucial role in DNA damage response (DDR) by hydroxylating TELO2, promoting its interaction with ATR which is required for activation of the ATR/CHK1/p53 pathway. Its target proteins are preferentially recognized via a LXXLAP motif.

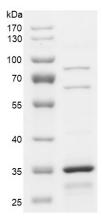
Protein Details: Recombinant human PHD3 (EGLN3) protein was expressed in *E.coli* as the full length protein (accession number NP_071356.1) with an N-terminal 6× His tag. The molecular weight of the protein is 31.1 kDa. **Assay Conditions:** 3 μM HIF1A (HIF-1α) peptide (DLDLEALAPYIPADDDFQL) was incubated with 300 nM PHD3 protein in 30 μl reaction system containing 20 mM Tris-HCl pH 7.5, 5 mM KCl, 1.5 mM MgCl2, 1 mM DTT, 100 μM 2-oxoglutarate, 100 μM ascorbate and 50 μM (NH4)2Fe(SO4)2·6H2O for 2 hours at 30°C. MALDI-TOF was used for detection.

Application Notes: This protein is suitable for use in binding assays, inhibitor screening, and selectivity profiling.

Assay Conditions: $3 \mu M$ HIF1A (HIF-1 α) peptide (DLDLEALAPYIPADDDFQL) was incubated with 300 nM PHD3 protein in 30 μ l reaction system containing 20 mM Tris-HCl pH 7.5, 5 mM KCl, 1.5 mM MgCl2, 1 mM DTT, 100 μ M 2-oxoglutarate, 100 μ M ascorbate and 50 μ M (NH4)2Fe(SO4)2·6H2O for 2 hours at 30°C. MALDI-TOF was used for detection.

Storage and Guarantee: Recombinant proteins in solution are temperature sensitive and must be stored at -80°C to prevent degradation. Avoid repeated freeze/thaw cycles and keep on ice when not in storage. This product is for research use only and is not for use in diagnostic procedures. This product is guaranteed for 6 months from date of arrival.

PHD3 / EGLN3

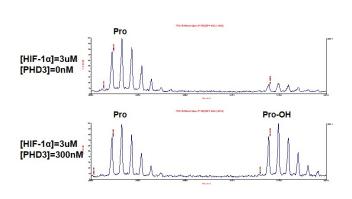


Recombinant PHD3 / EGLN3 protein

10% SDS-PAGE Coomassie staining.

MW: 31.1 kDa

Purity: >75%



MALDI-TOF Assay for Recombinant PHD3 / EGLN3 protein

 $3~\mu\text{M}$ HIF-1 α peptide was incubated with 300 nM PHD3 protein in 30 μI reaction system containing 20 mM Tris-HCl pH 7.5, 5 mM KCl, 1.5 mM MgCl2, 1 mM DTT, 100 μM 2-oxoglutarate, 100 μM ascorbate and 50 μM (NH4)2Fe(SO4) 2.6H2O for 2 hours at 30°C.

MALDI-TOF was used for detection.