Anti-HIF Prolyl Hydroxylases Antibody [JE63-25] HA722468

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IHC-P, IF-Cell, IP, FC
Molecular Wt:	Predicted band size: 47 kDa
Clone number:	JE63-25

Description: The product of this gene belongs to the family of prolyl 4-hydroxylases. This protein is a prolyl hydroxylase that may be involved in the degradation of hypoxia-inducible transcription factors under normoxia. It plays a role in adaptation to hypoxia and may be related to cellular oxygen sensing. Alternatively spliced variants encoding different isoforms have been identified. Catalyzes the post-translational formation of 4-hydroxyproline in hypoxia-inducible factor (HIF) alpha proteins. Hydroxylates HIF1A at 'Pro-402' and 'Pro-564'. 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. An autosomal recessive neurodevelopmental disorder characterized by global developmental delay, poor or absent speech, hypotonia, variable ocular movement and visual abnormalities, and respiratory difficulties. Disease onset is in infancy and death due to respiratory insufficiency may occur.

Immunogen: Synthetic peptide within Human HIF Prolyl Hydroxylases aa 51-150 / 502.

Positive control: HEK-293 cell lysate, A549 cell lysate, HCT 116 cell lysate, HeLa cell lysate, HEK-293, human lung cancer tissue, human colon cancer tissue.

Subcellular location: Endoplasmic reticulum membrane.

Database links: SwissProt: Q9NXG6 Human

Recommended Dilutions:

WB	1:1,000
IHC-P	1:200
IF-Cell	1:100
IP	1-2μg/sample
FC	1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

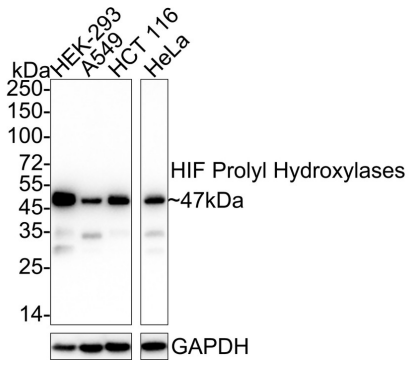

Fig1: Western blot analysis of HIF Prolyl Hydroxylases on different lysates with Rabbit anti-HIF Prolyl Hydroxylases antibody (HA722468) at 1/1,000 dilution.

Lane 1: HEK-293 cell lysate
Lane 2: A549 cell lysate
Lane 3: HCT 116 cell lysate
Lane 4: HeLa cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 47 kDa
Observed band size: 47 kDa

Exposure time: 25 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722468) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

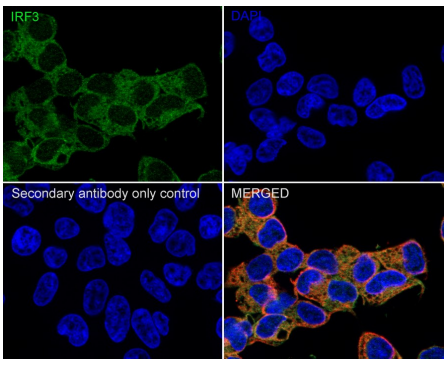

Fig2: Immunocytochemistry analysis of HEK-293 cells labeling HIF Prolyl Hydroxylases with Rabbit anti-HIF Prolyl Hydroxylases antibody (HA722468) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-HIF Prolyl Hydroxylases antibody (HA722468) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

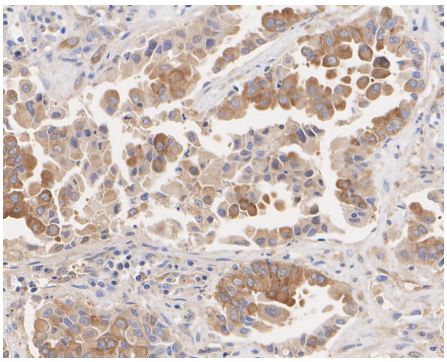

Fig3: Immunohistochemical analysis of paraffin-embedded human lung cancer tissue with Rabbit anti-HIF Prolyl Hydroxylases antibody (HA722468) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722468) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

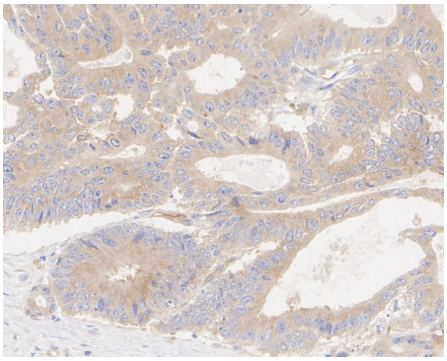

Fig4: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-HIF Prolyl Hydroxylases antibody (HA722468) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722468) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

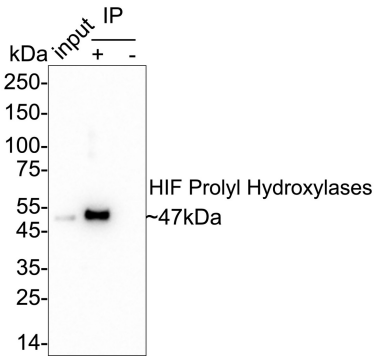

Fig5: HIF Prolyl Hydroxylases was immunoprecipitated from 0.2 mg HEK-293 cell lysate with HA722468 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA722468 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HEK-293 cell lysate (input)
Lane 2: HA722468 IP in HEK-293 cell lysate
Lane 3: Rabbit IgG instead of HA722468 in HEK-293 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 26 seconds; ECL: K1801

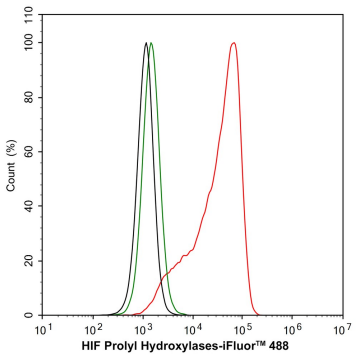

Fig6: Flow cytometric analysis of HEK-293 cells labeling HIF Prolyl Hydroxylases.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722468, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Koivunen P., Tiainen P., Hyvaerinen J., Williams K.E., Sormunen R., Klaus S.J., Kivirikko K.I., Myllyharju J. An endoplasmic reticulum transmembrane prolyl 4-hydroxylase is induced by hypoxia and acts on hypoxia-inducible factor alpha. J. Biol. Chem. 282:30544-30552 (2007)