Anti-HIF-1 alpha Antibody [PSH07-00]

HA722778

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IF-Cell, IHC-P
Molecular Wt:	Predicted band size: 93 kDa
Clone number:	PSH07-00

Description: Hypoxia-inducible factor 1-alpha, also known as HIF-1-alpha, is a subunit of a heterodimeric transcription factor hypoxia-inducible factor 1 (HIF-1) that is encoded by the HIF1A gene. The Nobel Prize in Physiology or Medicine 2019 was awarded for the discovery of HIF. HIF1A is a basic helix-loop-helix PAS domain containing protein, and is considered as the master transcriptional regulator of cellular and developmental response to hypoxia. The dysregulation and overexpression of HIF1A by either hypoxia or genetic alternations have been heavily implicated in cancer biology, as well as a number of other pathophysiologies, specifically in areas of vascularization and angiogenesis, energy metabolism, cell survival, and tumor invasion. Two other alternative transcripts encoding different isoforms have been identified.

Immunogen: Synthetic peptide within human HIF-1 alpha aa 441-490.

Positive control: HeLa cell lysate, HeLa treated with 0.5mM CoCl2 for 6 hours cell lysate, HepG2 cell lysate, HepG2 treated with 100μM CoCl2 for 4 hours cell lysate, C2C12 cell lysate, C2C12 treated with 100μM CoCl2 for 4 hours cell lysate, HeLa cells treated with 0.5mM CoCl2 for 6 hours, C2C12 cells treated with 200μM CoCl2 for 4 hours.

Subcellular location: Cytoplasm, Nucleus, Nucleus speckle.

Database links: SwissProt: Q16665 Human | Q61221 Mouse

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:100
IHC-P	1:1,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

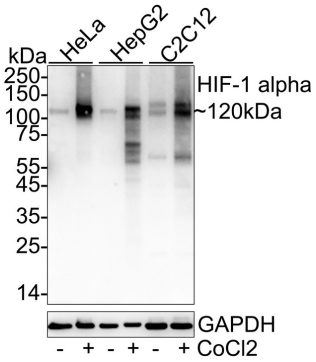

Fig1: Western blot analysis of HIF-1 alpha on different lysates with Rabbit anti-HIF-1 alpha antibody (HA722778) at 1/5,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 0.5mM CoCl2 for 6 hours cell lysate
Lane 3: HepG2 cell lysate
Lane 4: HepG2 treated with 100μM CoCl2 for 4 hours cell lysate
Lane 5: C2C12 cell lysate
Lane 6: C2C12 treated with 100μM CoCl2 for 4 hours cell lysate

Lysates/proteins at 30 μg/Lane.

Predicted band size: 93 kDa
Observed band size: 120 kDa

Exposure time: 20 seconds; ECL: K1802;

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 (HA722778) at 1/5,000 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.

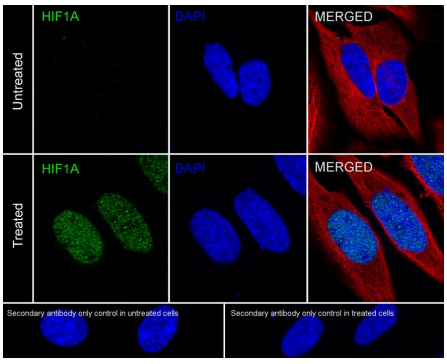

Fig2: Immunocytochemistry analysis of HeLa cells treated with 0.5mM CoCl2 for 6 hours labeling HIF-1 alpha with Rabbit anti-HIF-1 alpha antibody (HA722778) at 1/100 dilution.

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-1 alpha antibody (HA722778) 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.

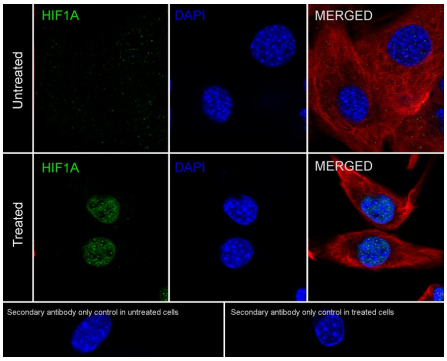

Fig3: Immunocytochemistry analysis of C2C12 cells treated with 200μM CoCl2 for 4 hours labeling HIF-1 alpha with Rabbit anti-HIF-1 alpha antibody (HA722778) at 1/100 dilution.

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-1 alpha antibody (HA722778) 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.

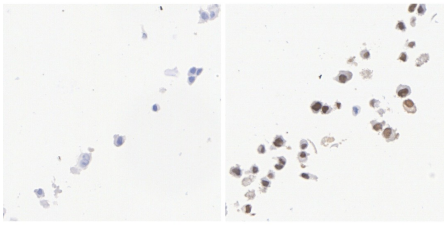

Fig4: Immunohistochemical analysis of paraffin-embedded HeLa cells treated with 0.5mM CoCl2 for 6 hours with Rabbit anti-HIF-1 alpha antibody (HA722778) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (HA722778) 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.

Background References

1. Korbecki J et al. Chronic and Cycling Hypoxia: Drivers of Cancer Chronic Inflammation through HIF-1 and NF-κB Activation: A Review of the Molecular Mechanisms. Int J Mol Sci. 2021 Oct
2. Infantino V et al. Cancer Cell Metabolism in Hypoxia: Role of HIF-1 as Key Regulator and Therapeutic Target. Int J Mol Sci. 2021 May