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Product datasheet

Anti-HIF-1 alpha antibody ab216842

38 References 5 Images

Overview

Product name	Anti-HIF-1 alpha antibody
Description	Rabbit polyclonal to HIF-1 alpha
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide within Human HIF-1 alpha aa 350-450 conjugated to keyhole limpet haemocyanin. The exact immunogen sequence used to generate this antibody is proprietary information. If additional detail on the immunogen is needed to determine the suitability of the antibody for your needs, please **contact** our Scientific Support team to discuss your requirements. Database link: **Q16665** Run BLAST with Run BLAST with
Positive control	WB: Hela, A431, U251, HepG2, HL60 and U-87MG cell lysate; ICC/IF: Hela cells. Flow Cyt (Intra): Mouse spleen cells.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Proclin 300 Constituents: 50% Glycerol (glycerin, glycerine), 1% BSA, 48.98% TBS, 1X
Purity	Protein A purified

Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab216842 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/300 - 1/1000. Detects a band of approximately 92 kDa (predicted molecular weight: 92 kDa). Ensure cell lysis occurs quickly if removed from hypoxia. Ultrasonic lysis is recommended to enrich more nuclear lysate. Positive Control: Hypoxic samples such as HeLa-DFO treated whole cell lysate **ab116322**. For a stronger signal, HeLa-DFO treated nuclear extracts are recommended **ab180880**. The cell
ICC/IF		1/50 - 1/200.
Flow Cyt (Intra)		1/20 - 1/100.

Target

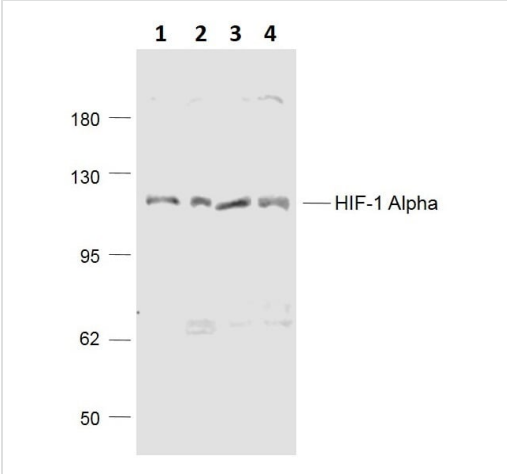
Function	Functions as a master transcriptional regulator of the adaptive response to hypoxia. Under hypoxic conditions activates the transcription of over 40 genes, including, erythropoietin, glucose transporters, glycolytic enzymes, vascular endothelial growth factor, and other genes whose protein products increase oxygen delivery or facilitate metabolic adaptation to hypoxia. Plays an essential role in embryonic vascularization, tumor angiogenesis and pathophysiology of ischemic disease. Binds to core DNA sequence 5'-[AG]CGTG-3' within the hypoxia response element (HRE) of target gene promoters. Activation requires recruitment of transcriptional coactivators such as CREBPB and EP300. Activity is enhanced by interaction with both, NCOA1 or NCOA2. Interaction with redox regulatory protein APEX seems to activate CTAD and potentiates activation by NCOA1 and CREBBP.
Tissue specificity	Expressed in most tissues with highest levels in kidney and heart. Overexpressed in the majority of common human cancers and their metastases, due to the presence of intratumoral hypoxia and as a result of mutations in genes encoding oncoproteins and tumor suppressors.
Sequence similarities	Contains 1 basic helix-loop-helix (bHLH) domain. Contains 1 PAC (PAS-associated C-terminal) domain. Contains 2 PAS (PER-ARNT-SIM) domains.
Domain	Contains two independent C-terminal transactivation domains, NTAD and CTAD, which function synergistically. Their transcriptional activity is repressed by an intervening inhibitory domain (ID).
Post-translational modifications	In normoxia, is hydroxylated on Pro-402 and Pro-564 in the oxygen-dependent degradation domain (ODD) by EGLN1/PHD1 and EGLN2/PHD2. EGLN3/PHD3 has also been shown to hydroxylate Pro-564. The hydroxylated prolines promote interaction with VHL, initiating rapid ubiquitination and subsequent proteasomal degradation. Deubiquitinated by USP20. Under hypoxia, proline hydroxylation is impaired and ubiquitination is attenuated, resulting in stabilization.

In normoxia, is hydroxylated on Asn-803 by HIF1AN, thus abrogating interaction with CREBBP and EP300 and preventing transcriptional activation. This hydroxylation is inhibited by the Cu/Zn-chelator, Clioquinol.
S-nitrosylation of Cys-800 may be responsible for increased recruitment of p300 coactivator necessary for transcriptional activity of HIF-1 complex.
Requires phosphorylation for DNA-binding.
Sumoylated; by SUMO1 under hypoxia. Sumoylation is enhanced through interaction with RWDD3. Desumoylation by SENP1 leads to increased HIF1A stability and transriptional activity.
Ubiquitinated; in normoxia, following hydroxylation and interaction with VHL. Lys-532 appears to be the principal site of ubiquitination. Clioquinol, the Cu/Zn-chelator, inhibits ubiquitination through preventing hydroxylation at Asn-803.
The iron and 2-oxoglutarate dependent 3-hydroxylation of asparagine is (S) stereospecific within HIF CTAD domains.

Cellular localization

Cytoplasm. Nucleus. Cytoplasmic in normoxia, nuclear translocation in response to hypoxia. Colocalizes with SUMO1 in the nucleus, under hypoxia.

Images



Western blot - Anti-HIF-1 alpha antibody (ab216842)

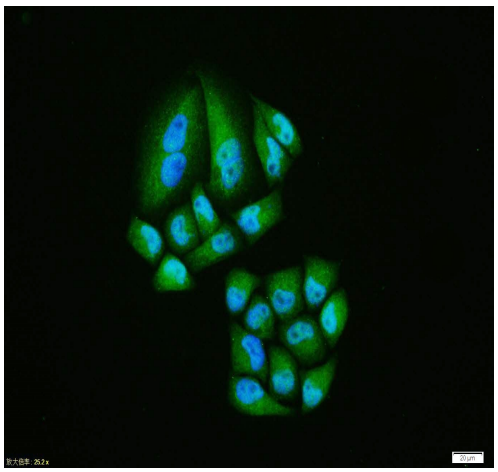
All lanes : Anti-HIF-1 alpha antibody (ab216842) at 1/1000 dilution

Lane 1 : Hela cell lysate
Lane 2 : A431 cell lysate
Lane 3 : U251 cell lysate
Lane 4 : HepG2 cell lysate

Secondary
All lanes : Conjugated secondary antibody at 1/20000 dilution

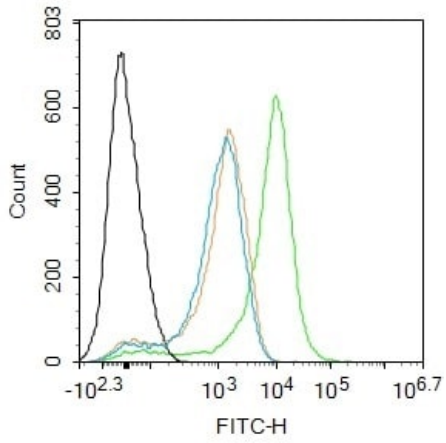
Predicted band size: 92 kDa

Cell lysates were first probed with primary antibody (ab216842) at 4°C overnight then incubated with conjugated secondary antibody for 60 min at 37°C.



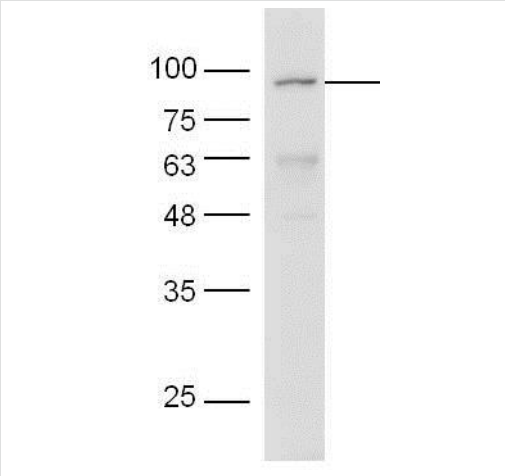
Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody (ab216842)

Hela cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum) at 37°C for 20 min; Antibody incubation with HIF-1 Alpha polyclonal Antibody (1/100, ab216842), 90 minutes at 37°C; followed by a conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue) was used to stain the cell nuclei.



Flow Cytometry (Intracellular) - Anti-HIF-1 alpha antibody (ab216842)

Mouse spleen cells were fixed with 4% PFA for 10min at room temperature, permeabilized with 90% ice-cold methanol for 20 min at -20I, and incubated in 5% BSA blocking buffer for 30 min at room temperature. Cells were then stained with HIF-1 Alpha Antibody (ab216842) at 1:50 dilution in blocking buffer and incubated for 30 min at room temperature, washed twice with 2%BSA in PBS, followed by secondary antibody incubation for 40 min at room temperature. Acquisitions of 20,000 events were performed. Cells stained with primary antibody (green), and isotype control (orange).



Western blot - Anti-HIF-1 alpha antibody (ab216842)

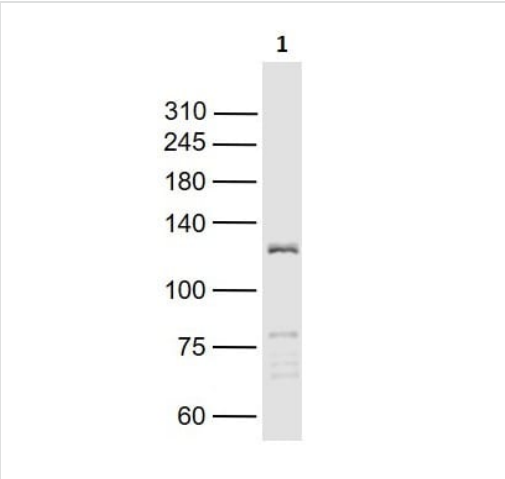
Anti-HIF-1 alpha antibody (ab216842) at 1/300 dilution + HL60 cell lysate

Secondary

Goat Anti-Rabbit IgG Antibody (H+L), HRP at 1/5000 dilution

Predicted band size: 92 kDa

Cell lysate was first probed with primary antibody (ab216842) at 4°C overnight then incubated with conjugated secondary antibody for 90 min at 37°C.



Western blot - Anti-HIF-1 alpha antibody (ab216842)

Anti-HIF-1 alpha antibody (ab216842) at 1/1000 dilution + U-87MG cell lysate

Secondary

Conjugated secondary antibody at 1/20000 dilution

Predicted band size: 92 kDa

Cell lysate was first probed with primary antibody (ab216842) at 4°C overnight then incubated with conjugated secondary antibody for 60 min at 37°C.

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