abcam

Product datasheet

Anti-HIF-1 alpha antibody [H1alpha67] ab1

44 Abreviews 340 References 5 Images

Overview

Product name	Anti-HIF-1 alpha antibody [H1alpha67]
Description	Mouse monoclonal [H1alpha67] to HIF-1 alpha
Host species	Mouse
Tested applications	**Suitable for:** IP, ICC/IF, WB, Flow Cyt (Intra) **Unsuitable for:** IHC-Fr or IHC-P
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa (DFO treated 0.5mM, 24h) nuclear lysate (**ab180880**); Human whole cell lysate (human lung adenocarcinoma cell line ADLC-5M2) (DFO treated 100uM, 16h). IP: HeLa (DFO treated 0.5 mg) nuclear lysate. ICC/F: MCF7 cells. Flow Cyt (Intra): HeLa (Human epithelial cell line from cervix adenocarcinoma) cells treated with 1mM Deferoxamine (**ab120727**) for 24 hours.
General notes	**For WB, we recommend using positive control samples such as DFO or CoCl2 treated nulcear cell lysates such as ab180880. Ensure cell lysis occurs quickly (within 2 mins) if removed from hypoxia. Loading a high amount of sample (>50 µg) and addition of protease inhibitors (e.g. ab65621) may also enhance detection.** This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. 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. Avoid freeze / thaw cycle.

Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Purity	Protein G purified
Clonality	Monoclonal
Clone number	H1alpha67
Isotype	IgG2b

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab1 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
IP	★★★★☆ **(2)**	Use a concentration of 5 µg/ml.
ICC/IF	★★★★☆ **(6)**	1/100 - 1/200. PubMed: 25422886 We recommend **Goat Anti-Mouse IgG H&L (DyLight® 488) preadsorbed (ab96879) secondary antibody**.
WB	★★★★☆ **(22)**	Use a concentration of 5 µg/ml. Detects a band of approximately 120 kDa (predicted molecular weight: 92 kDa). We recommend blocking for 1 hour with 5% milk in TBST and reducing to 2% milk in TBST for the primary and secondary antibody incubation steps. For primary antibody incubation, we recommend 2 hours at room temperature. We recommend Goat Anti-Mouse IgG H&L (HRP) preadsorbed (**ab97040**) secondary
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab170192** - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.

Application notes Is unsuitable for IHC-Fr or IHC-P.

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).

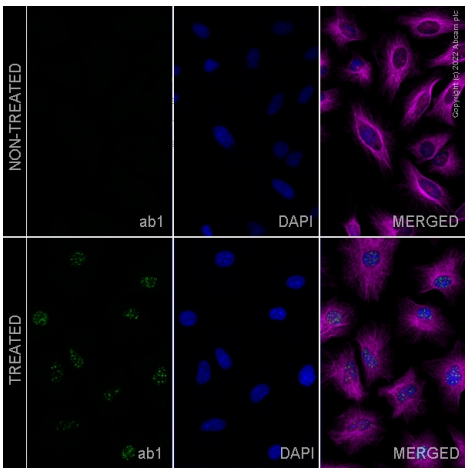
Post-translational modifications

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



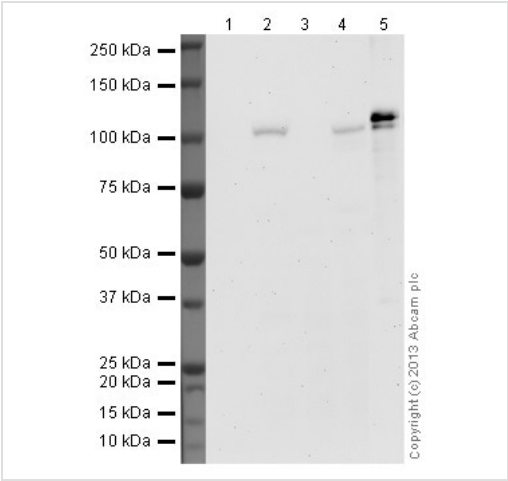
Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [H1alpha67] (ab1)

ab1 staining HIF-1 alpha in HeLa DFO cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab1 at 10µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour magenta). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal

sections is shown.



Western blot - Anti-HIF-1 alpha antibody [H1alpha67] (ab1)

All lanes : Anti-HIF-1 alpha antibody [H1alpha67] (ab1) at 5 µg/ml

Lane 1 : HeLa nuclear extract lysate (**ab150036**) at 40 µg
Lane 2 : Hela-DFO treated (0.5mM, 24h) Nuclear Lysate (**ab180880**) at 40 µg
Lane 3 : HeLa nuclear control at 40 µg
Lane 4 : HeLa nuclear DFO treated at 40 µg
Lane 5 : Recombinant Human HIF-1 alpha protein (**ab154478**) at 0.001 µg

Secondary
All lanes : Goat Anti-Mouse IgG H&L (HRP) preadsorbed (**ab97040**) at 1/10000 dilution

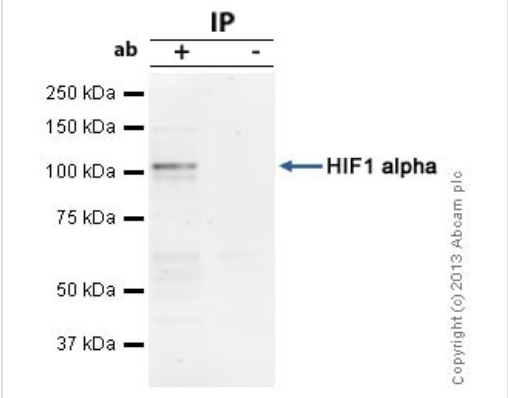
Performed under reducing conditions.

Predicted band size: 92 kDa

Exposure time: 20 minutes

We recommend using 5% milk in TBST as the blocking agent, decreasing to 2% milk in TBST during primary and secondary antibody incubation.

Blots were developed with **Goat Anti-Mouse IgG H&L (HRP) preadsorbed (ab97040) secondary antibody**



Immunoprecipitation - Anti-HIF-1 alpha antibody [H1alpha67] (ab1)

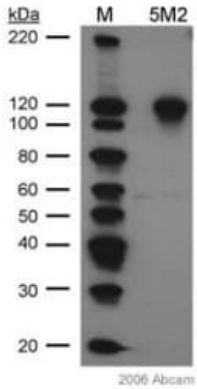
HIF-1 alpha was immunoprecipitated using 0.5 mg HeLa Nuclear DFO treated whole cell extract (**ab180880**), 5 µg of Mouse monoclonal to HIF-1 alpha and 50 µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10 minutes, HeLa DFO treated whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10 minutes under agitation.

Proteins were eluted by addition of 40 µl SDS loading buffer and incubated for 10 minutes at 70°C; 10 µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab1.

Secondary: Goat polyclonal to mouse IgG light chain specific (HRP) at 1:20,000 dilution.

Band: 110 kDa; HIF1 alpha



Western blot - Anti-HIF-1 alpha antibody [H1alpha67] (ab1)

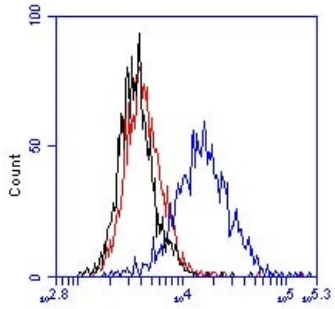
This image is taken from an Abreview submitted by Mike Campa, no further information is known about this image

Anti-HIF-1 alpha antibody [H1alpha67] (ab1) at 1/400 dilution + Human whole cell lysate (human lung adenocarcinoma cell line ADLC-5M2) treated for 16 hours with 100 micromolar deferoxamine (DFO) at 20 µg

Performed under reducing conditions.

Predicted band size: 92 kDa
Observed band size: 120 kDa

PVDF membrane was used and blocked for 16 hours in 5% milk.



Flow Cytometry (Intracellular) - Anti-HIF-1 alpha antibody [H1alpha67] (ab1)

Flow cytometry using ab1. HeLa (Human epithelial cell line from cervix adenocarcinoma) cells were cultured untreated or with 1mM Deferoxamine (**ab120727**) for 24 hours to induce HIF-1-alpha protein levels. Cells were then trypsinized, fixed with paraformaldehyde and stained with ab1 (0.5 µg/mL). 1% BSA in PBS was used as the blocking buffer throughout. ab1 was labeled with and anti-mouse Alexa-Fluor® 488 dye. Unstained (black), untreated (red) and DFO treated (blue) cell traces are shown.

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