abcam

Product datasheet

Anti-HIF-1 alpha antibody - ChIP Grade ab2185

10 Abreviews 84 References 5 Images

Overview

Product name	Anti-HIF-1 alpha antibody - ChIP Grade
Description	Rabbit polyclonal to HIF-1 alpha - ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, WB, IP, IHC-Fr, ICC, ICC/IF, ChIP
Species reactivity	**Reacts with:** Mouse, Rat, Guinea pig, Human, Xenopus laevis, Monkey **Predicted to work with:** Cow
Immunogen	Fusion protein corresponding to Human HIF-1 alpha aa 432-528.
General notes	HIF-1 alpha can be a difficult target to work with so we have compiled a summary of all the important information you need to know including useful tips. This can be found in the protocols tab or alternatively click here to download it. Under normoxic conditions HIF-1 alpha has a short half-life. It is largely undetectable in cells or tissues grown under normoxic conditions. It is stabilized only at O_2 concentrations below 5% and upon stabilization under hypoxic conditions HIF-1 translocates to the nucleus. Therefore we recommend western blots using nuclear extracts and running Hypoxia treated samples as positive control (ab180880). Hypoxia can be induced with treatment using certain agents e.g. $CoCl_2$ or DFO, etc. so proper sample preparation is critical.

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	Preservative: 0.05% Sodium azide Constituents: Tris glycine, 0.87% Sodium chloride
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab2185** 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
IHC-P	★★★★★	1/100.
WB	★★★★☆	1/500 - 1/1000.
IP	★★★☆☆	1/1000.
IHC-Fr		1/10 - 1/2000.
ICC	★★★★☆	Use at an assay dependent concentration.
ICC/IF	★★★★★	1/10 - 1/2000.
EMSA		1/1 - 1/100.
ChIP	★★★★★	Use at an assay dependent concentration. 25 µl / 15 millions cells

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

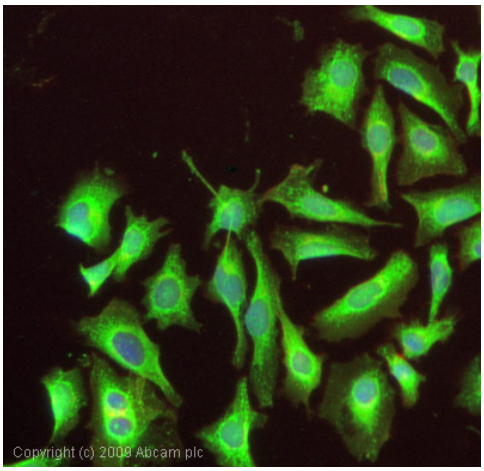
In normoxia, is hydroxylated on Pro-402 and Pro-564 in the oxygen-dependent degradation domain (ODD) by EGLN1/PHD1 and EGLN2/PHD2. EGLN3/PHD3 has also been shown to hydroxylate Pro-564. The hydroxylated prolines promote interaction with VHL, initiating rapid ubiquitination and subsequent proteasomal 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

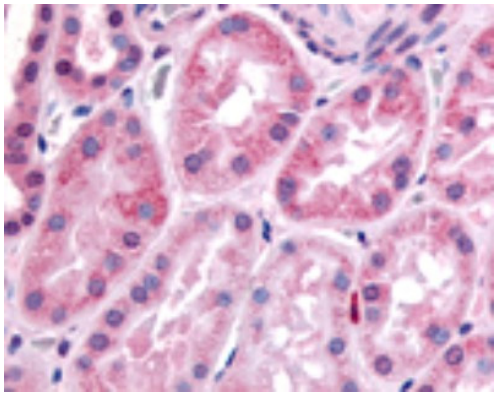
Cytoplasm. Nucleus. Cytoplasmic in normoxia, nuclear translocation in response to hypoxia. Colocalizes with SUMO1 in the nucleus, under hypoxia.

Images



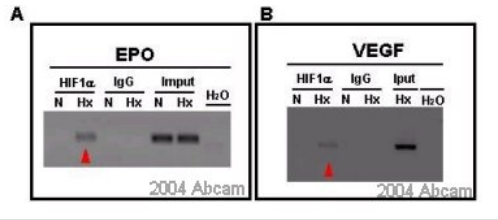
Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody - ChIP Grade (ab2185)

ICC/IF image of ab2185 stained HeLa cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab2185, 1/1000 dilution) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody - ChIP Grade (ab2185)

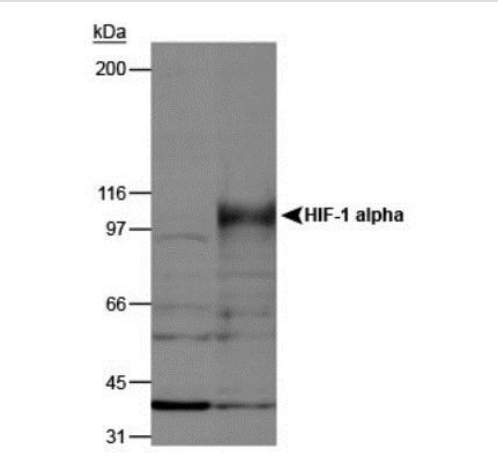
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human kidney tissue sections labeling HIF-1-alpha with ab2185.



ChIP - Anti-HIF-1 alpha antibody - ChIP Grade (ab2185)

This image is courtesy of a review submitted by Yolanda Cuevas, Hospital La Princesa.

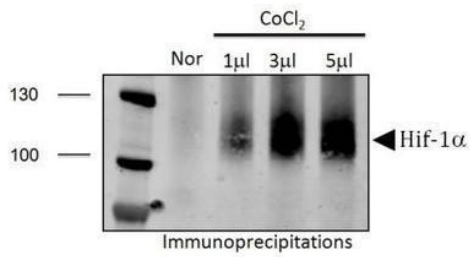
ChIP analysis of HIF-1-alpha genomic sequences from HeLa cells cultivated in normoxic (N) or hypoxic (Hx) conditions, using a HIF1-alpha polyclonal antibody (ab2185). For a negative control, IgG was used and the input as a positive control in the subsequent PCR. Primers for known target genes were used HIF1 alpha, A. EPO and B. VEGF.



Western blot - Anti-HIF-1 alpha antibody - ChIP Grade (ab2185)

All lanes : Anti-HIF-1 alpha antibody - ChIP Grade (ab2185)

Lane 1 : Rat nuclear extract lysate - normoxic
Lane 2 : Rat nuclear extract lysate - hypoxic



Immunoprecipitation - Anti-HIF-1 alpha antibody - ChIP Grade (ab2185)

All lanes : Anti-HIF-1 alpha antibody - ChIP Grade (ab2185)

Lane 1 : PC-3 cell lysates - untreated
Lane 2 : PC-3 cell lysates - treated with 1µl cobalt chloride
Lane 3 : PC-3 cell lysates - treated with 3µl cobalt chloride
Lane 4 : PC-3 cell lysates - treated with 5µl cobalt chloride

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