abcam

Product datasheet

Anti-HIF-1 alpha antibody [BL-124-3F7] - BSA free ab272040

Recombinant

6 Images

Overview

Product name	Anti-HIF-1 alpha antibody [BL-124-3F7] - BSA free
Description	Rabbit monoclonal [BL-124-3F7] to HIF-1 alpha - BSA free
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP, ChIP-sequencing, ICC, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment corresponding to Human HIF-1 alpha aa 433-826. NP_001521.1 and Gene ID 3091. Database link: **Q16665**
Positive control	ChIPseq: CoCl2 treated Hep-G2 cell lysate. IHC-P: Human prostate carcinoma tissue. IP: CoCl2 treated Hep-G2 and HeLa cell lysates. ICC: CoCl2 treated HepG2 cells.
General notes	ab272040 is the BSA-free version of **ab243860**. This product is sold under License from Bethyl Laboratories, Inc.

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: 8.20 Preservative: 0.09% Sodium azide Constituent: 98% Borate buffered saline
Purification notes	Recombinant antibody was purified from cell culture supernatant.
Clonality	Monoclonal
Clone number	BL-124-3F7
Isotype	IgG

Applications

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab272040 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		Use at an assay dependent concentration. 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 fractionation kit can also be purchased separately **ab109719**
IP		Use at an assay dependent concentration.
ChIP-sequencing		Use at an assay dependent concentration.
ICC		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol. Antigen retrieval with citrate buffer pH 6.0 for 20 minutes using a pressure cooker is recommended.

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).

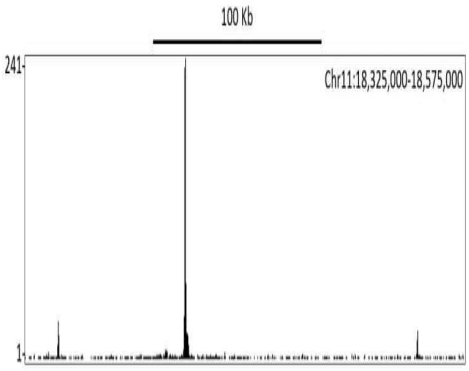
Post-translational modifications In normoxia, is hydroxylated on Pro-402 and Pro-564 in the oxygen-dependent degradation domain (ODD) by EGLN1/PHD1 and EGLN2/PHD2. EGLN3/PHD3 has also been shown to hydroxylate Pro-564. The hydroxylated prolines promote interaction with VHL, initiating rapid ubiquitination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

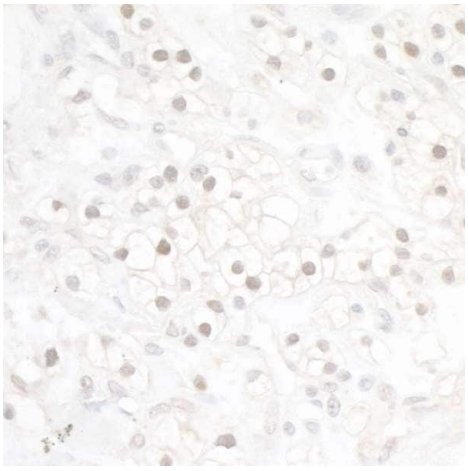
Cytoplasm. Nucleus. Cytoplasmic in normoxia, nuclear translocation in response to hypoxia. Colocalizes with SUMO1 in the nucleus, under hypoxia.

Images



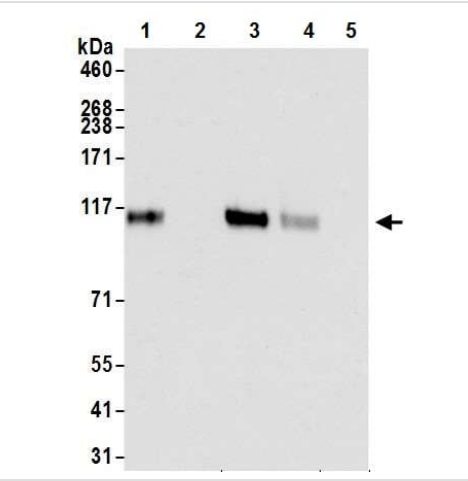
ChIP-sequencing - Anti-HIF-1 alpha antibody [BL-124-3F7] - BSA free (ab272040)

Localization of human HIF1-alpha binding sites in immunoprecipitates from CoCl2 treated Hep-G2 lysate by ChIP-Seq using **ab243860**
This data was developed using the same antibody clone in a different buffer formulation containing Borate buffered saline, BSA, glycerol and sodium azide (**ab243860**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [BL-124-3F7] - BSA free (ab272040)

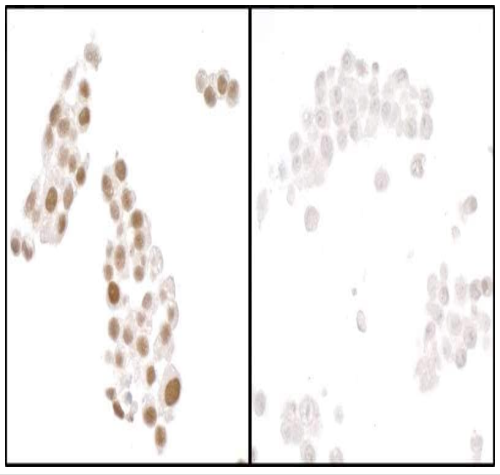
Formalin-fixed, paraffin-embedded human renal cell carcinoma tissue stained for HIF-1 alpha using **ab243860** at 1/100 dilution in immunohistochemical analysis. A HRP-conjugated goat anti-rabbit IgG was used as the secondary. DAB staining.
This data was developed using the same antibody clone in a different buffer formulation containing Borate buffered saline, BSA, glycerol and sodium azide (**ab243860**).



Immunoprecipitation - Anti-HIF-1 alpha antibody [BL-124-3F7] - BSA free (ab272040)

HIF-1 alpha was immunoprecipitated from 1mg CoCl2 and mock treated HepG2 and HeLa whole cell lysates with **ab243860** at 20µg/mL lysate.

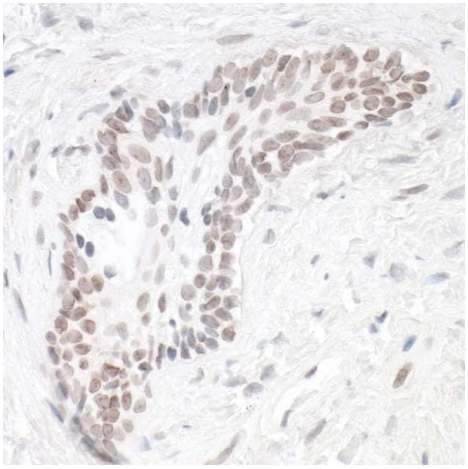
This data was developed using the same antibody clone in a different buffer formulation containing Borate buffered saline, BSA, glycerol and sodium azide (**ab243860**).



Immunocytochemistry - Anti-HIF-1 alpha antibody [BL-124-3F7] - BSA free (ab272040)

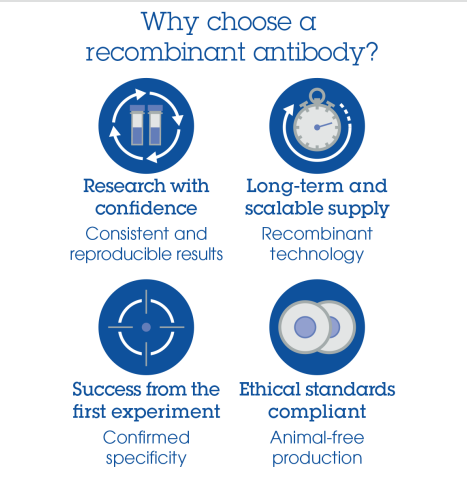
Formalin-fixed, paraffin-embedded HepG2 (human liver hepatocellular carcinoma cell line) cells labeling HIF-1 alpha using **ab243860** at 1/100 dilution in ICC analysis. A HRP-conjugated goat-anti rabbit IgG was used as the secondary. DAB staining.

This data was developed using the same antibody clone in a different buffer formulation containing Borate buffered saline, BSA, glycerol and sodium azide (**ab243860**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [BL-124-3F7] - BSA free (ab272040)

Formalin-fixed, paraffin-embedded human prostate carcinoma tissue stained for HIF-1 alpha using **ab243860** at 1/100 dilution in immunohistochemical analysis. A HRP-conjugated goat anti-rabbit IgG was used as the secondary. DAB staining.
This data was developed using the same antibody clone in a different buffer formulation containing Borate buffered saline, BSA, glycerol and sodium azide (**ab243860**).



Anti-HIF-1 alpha antibody [BL-124-3F7] - BSA free (ab272040)

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