abcam

Product datasheet

Anti-HIF-1 alpha antibody [EPR16897] ab179483

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-HIF-1 alpha antibody [EPR16897]
Description	Rabbit monoclonal [EPR16897] to HIF-1 alpha
Host species	Rabbit
Specificity	Stimulation is required for the detection of HIF-1 alpha in most samples. For better results in WB, please try 1% SDS Hot lysis method presented in applications section.
Tested applications	**Suitable for:** ChIP-sequencing, WB, ICC/IF, ChIC/CUT&RUN-seq
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Sheep, Rabbit, Cow, Ferret, Non human primates
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1 treated with DMOD, HeLa treated with DMOG, HeLa treated with CoCl2; NIH/3T3 treated with CoCl2, and C6 treated with CoCl2 and MG-132 (**ab141003**) cell lysates. ICC/IF: HeLa cells treated with DFO (1 mM, 24 h). ChIP-Seq: HeLa cells. ChIC/CUT&RUN-Seq: HeLa cells.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

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	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR16897
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab179483 in the following tested applications.

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

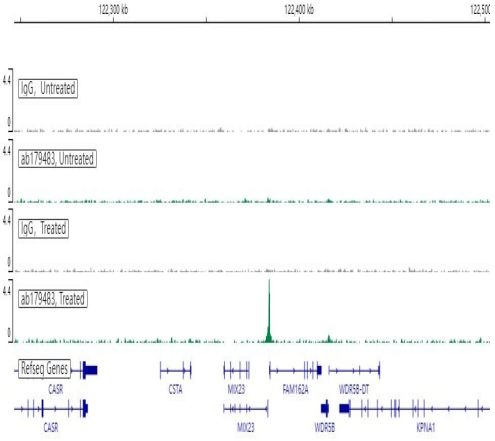
Application	Abreviews	Notes
ChIP-sequencing		Use 8µg for 10^7 cells.
WB	★★★★☆ **(5)**	1/1000. Detects a band of approximately 110 kDa (predicted molecular weight: 92 kDa). This antibody (ab179483) has been confirmed for **Mouse sample in WB.** This antibody works better in 1% SDS Hot Lysates in WB. For Lysate preparation protocol, please refer to the protocol book in the protocol section and/or **here (downloadable copy)**
ICC/IF	★★★★★ **(1)**	1/500.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5 µg

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	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

modifications	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

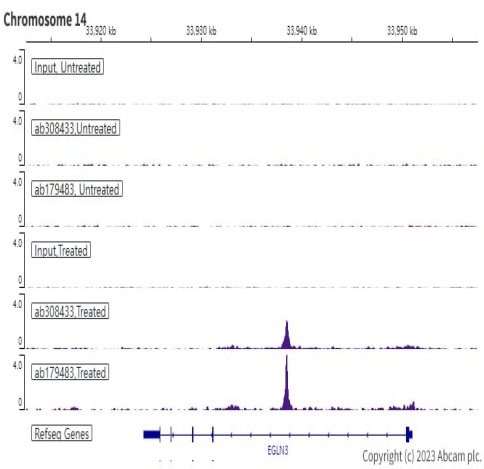


ChIC/CUT&RUN sequencing - Anti-HIF-1 alpha antibody [EPR16897] (ab179483)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2.5 x 10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells treated with Cocl2 (500 µM 20h+4h) and MG-132 (10µM 4h) and 5 µg of ab179483 [EPR16897]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown.

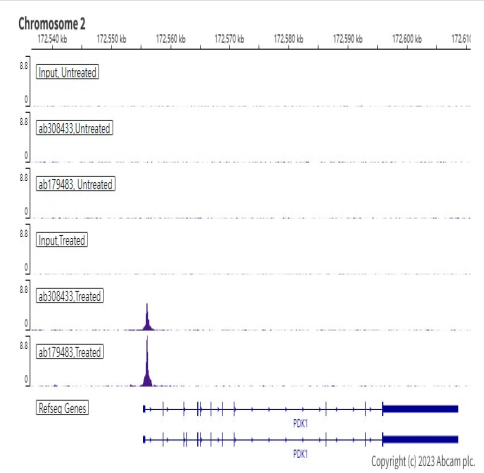
Additional screenshots of mapped reads can be downloaded **here**.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



ChIP-sequencing - Anti-HIF-1 alpha antibody [EPR16897] (ab179483)

Chromatin was prepared from HeLa cells treated with CoCl2 (350µM 20h+4h) add MG-132(10µM 4h). Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 cells and 8 µg of **ab308433** [EPR16897-145] or ab179483 [EPR16897]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads. The Input control is also shown.



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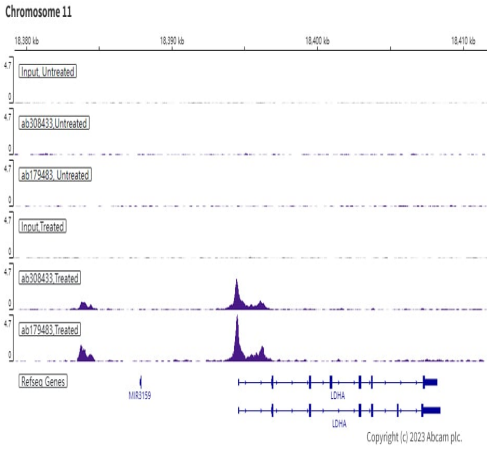

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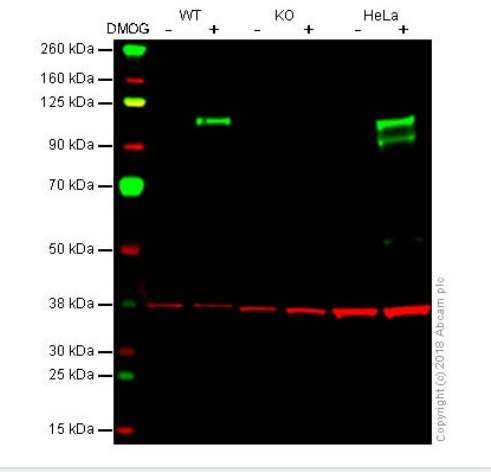


Western blot - Anti-HIF-1 alpha antibody [EPR16897] (ab179483)

All lanes : Anti-HIF-1 alpha antibody [EPR16897] (ab179483) at 1/1000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : Wild type HAP1 treated with DMOG (0.5mM 18hr) whole cell lysate
Lane 3 : HIF1A knockout HAP1 whole cell lysate
Lane 4 : HIF1A knockout HAP1 treated with DMOG (0.5mM 18hr) whole cell lysate
Lane 5 : HeLa whole cell lysate
Lane 6 : HeLa treated with DMOG (0.5mM 18hr) whole cell lysate

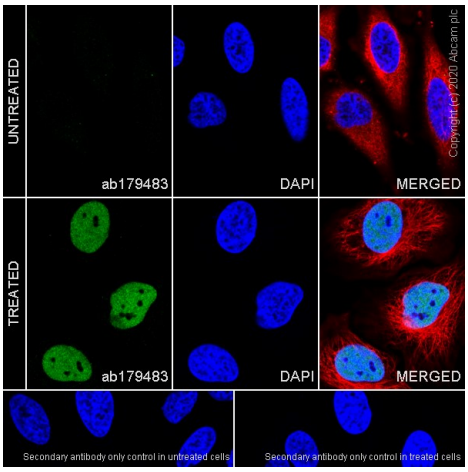
Lysates/proteins at 40 µg per lane.

Predicted band size: 92 kDa
Observed band size: 105 kDa

Lanes 1 - 6: Merged signal (red and green). Green - ab179483 observed at 105 kDa. Red - loading control, **ab24834**, observed at 50 kDa.

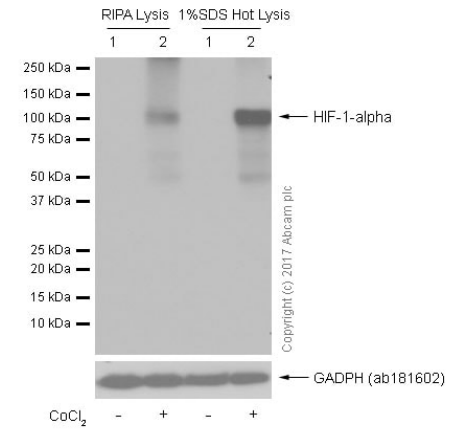
ab179483 was shown to specifically react with HIF-1 alpha in wild-

type HAP1 treated DMOG (0.5mM 18hr) cells as signal was lost in HAP1 knockout treated DMOG (0.5 mM 18hr) knockout cells. Wild-type and HAP1 knockout samples were subjected to SDS-PAGE. ab179483 and **ab24834** (Mouse anti-Histone H3 loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [EPR16897] (ab179483)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling HIF-1-alpha with ab179483 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining in HeLa cells treated with DFO (1 mM, 24 h). The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab195889** (anti-alpha Tubulin mouse mAb) (Alexa Fluor® 594) at 1/200 dilution (red).



Western blot - Anti-HIF-1 alpha antibody [EPR16897] (ab179483)

All lanes : Anti-HIF-1 alpha antibody [EPR16897] (ab179483) at 1/5000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) treated with 0.5 mM CoCl2 for 6 hours whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

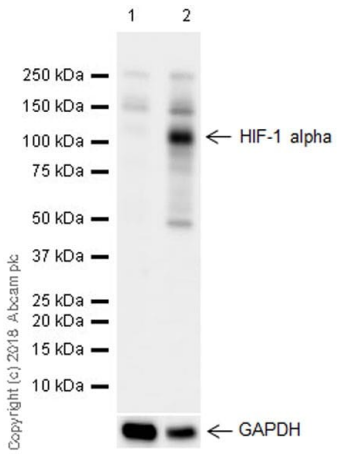
Predicted band size: 92 kDa
Observed band size: 110 kDa

Exposure time: 1 second

Blocking and diluting buffer: 5% NFDM/TBST

The different result is due to the lysates preparation method. The lysate in the left image is prepared by RIPA lysis method. The lysate in the right image is prepared by 1%SDS Hot lysis method. This antibody works better in 1%SDS Hot Lysates in WB.

For Lysate preparation protocol, please refer to the protocol book in the protocol section and/or **here (downloadable copy).**



Western blot - Anti-HIF-1 alpha antibody [EPR16897] (ab179483)

All lanes : Anti-HIF-1 alpha antibody [EPR16897] (ab179483) at 0.163 µg/ml

Lane 1 : Untreated C6 (rat glial tumor glial cell), whole cell lysate
Lane 2 : C6 treated with 400 µM CoCl2 and 20 µM MG-132 (**ab141003**) for 4 hours

Lysates/proteins at 10 µg per lane.

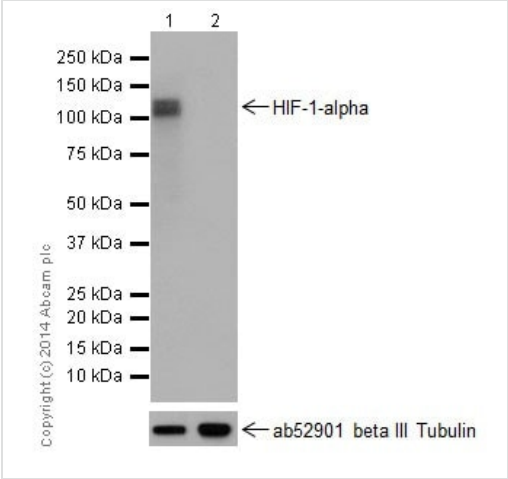
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 92 kDa
Observed band size: 110 kDa

Exposure time: 26 seconds

Blocking and diluting buffer: 5% NFDM/TBST.

The expression of HIF-1 alpha is induced by CoCl2 and maintained by MG-132 (PMID: 15836611).



Western blot - Anti-HIF-1 alpha antibody [EPR16897] (ab179483)

All lanes : Anti-HIF-1 alpha antibody [EPR16897] (ab179483) at 1/1000 dilution

Lane 1 : HeLa (Human epithelial cells from cervix adenocarcinoma) cell lysate treated with 0.5mM CoCl2 (Cobalt(II) chloride) for 6 hours.
Lane 2 : Untreated HeLa cell lysate

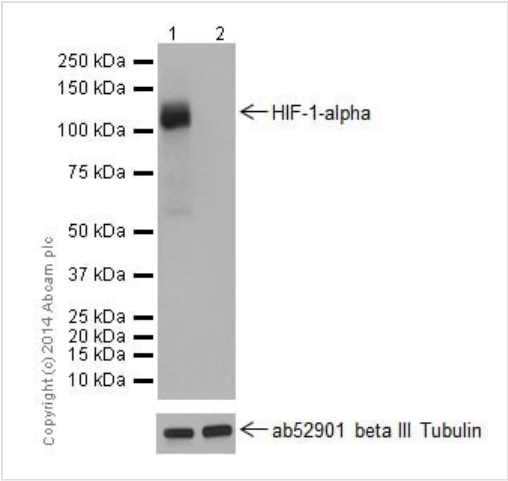
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 92 kDa
Observed band size: 110 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-HIF-1 alpha antibody [EPR16897] (ab179483)

All lanes : Anti-HIF-1 alpha antibody [EPR16897] (ab179483) at 1/1000 dilution

Lane 1 : NIH/3T3 (Mouse embyro fibroblast cells) cell lysate treated with 0.1mM CoCl2 for 24 hours.
Lane 2 : untreated NIH/3T3 cell lysate

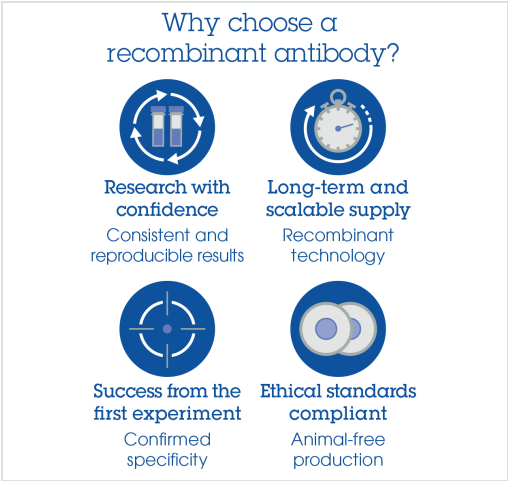
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 92 kDa
Observed band size: 110 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Anti-HIF-1 alpha antibody [EPR16897] (ab179483)

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