


Anti-HIF-1 alpha antibody [EPR16897] ab179483

KO 評価済 リコンビナント RabMAb

★★★★☆ 7 Abreviews 134 References 画像数 11

製品の概要

製品名	Anti-HIF-1 alpha antibody [EPR16897]
製品の詳細	Rabbit monoclonal [EPR16897] to HIF-1 alpha
由来種	Rabbit
特異性	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.
アプリケーション	適用あり: ChIP-sequencing, WB, ICC/IF, ChIC/CUT&RUN-seq
種交差性	交差種: Mouse, Rat, Human 交差が予測される動物種: Sheep, Rabbit, Cow, Ferret, Non human primates 
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: HAP1 treated with DMOD, HeLa treated with DMOG, HeLa treated with CoCl ₂ ; NIH/3T3 treated with CoCl ₂ , and C6 treated with CoCl ₂ 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.
特記事項	This product is a recombinant monoclonal antibody, which offers several advantages including: <ul style="list-style-type: none"> - 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 .

製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR16897
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab179483の使用に適用されます
 アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
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

ターゲット情報

機能	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.
組織特異性	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.
配列類似性	Contains 1 basic helix-loop-helix (bHLH) domain. Contains 1 PAC (PAS-associated C-terminal) domain. Contains 2 PAS (PER-ARNT-SIM) domains.
ドメイン	Contains two independent C-terminal transactivation domains, NTAD and CTAD, which function synergistically. Their transcriptional activity is repressed by an intervening inhibitory domain (ID).
翻訳後修飾	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 transcriptional activity.

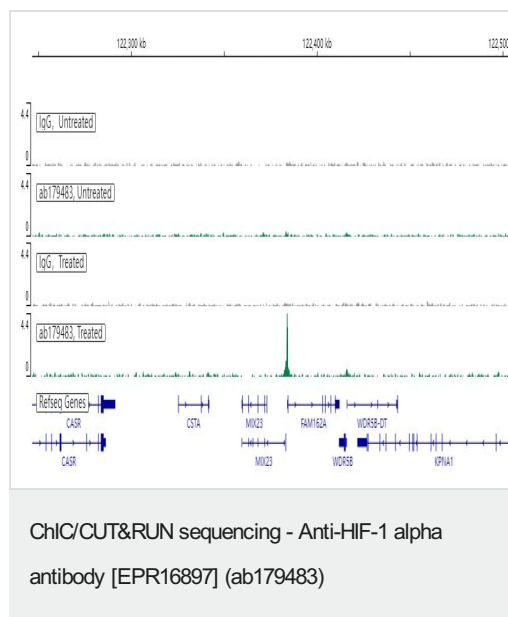
Ubiquitinated; in normoxia, following hydroxylation and interaction with VHL. Lys-532 appears to be the principal site of ubiquitination. Clioquinol, the Cu/Zn-chelator, inhibits ubiquitination through preventing hydroxylation at Asn-803.

The iron and 2-oxoglutarate dependent 3-hydroxylation of asparagine is (S) stereospecific within HIF CTAD domains.

細胞内局在

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

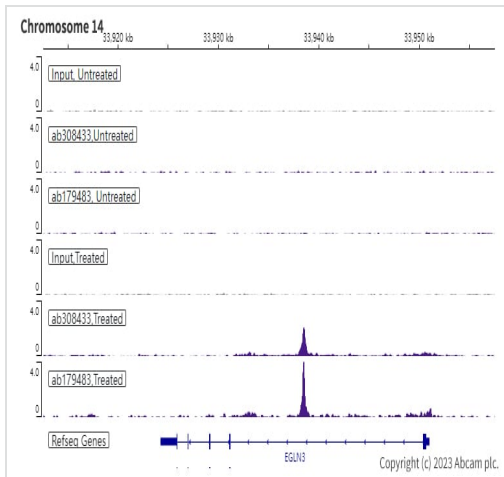
画像



ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL, 2.5 x 10⁵ 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.

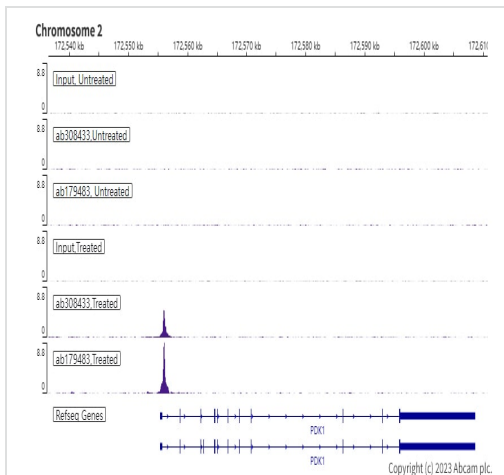
Additional screenshots of mapped reads can be downloaded [here](#).

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



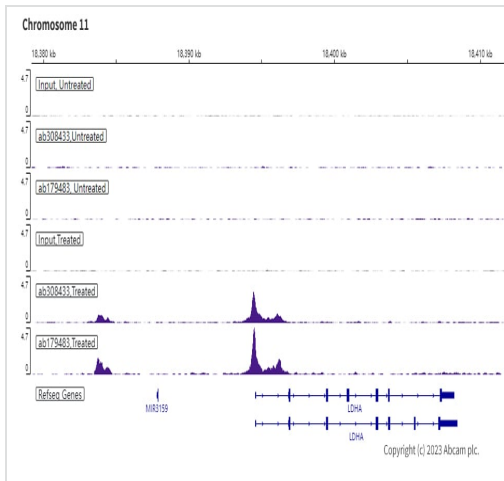
ChIP-sequencing - Anti-HIF-1 alpha antibody
[EPR16897] (ab179483)

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 10⁷ 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.



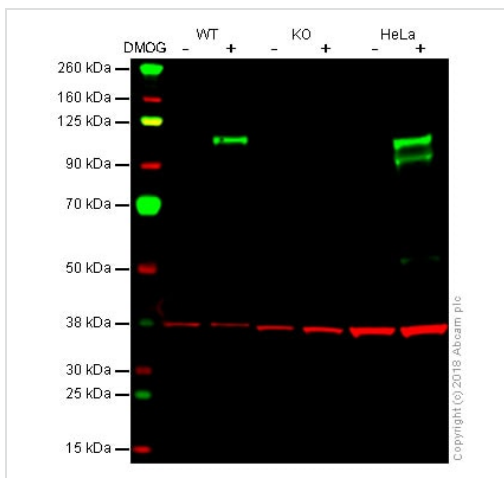
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Western blot - Anti-HIF-1 alpha antibody
[EPR16897] (ab179483)

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.

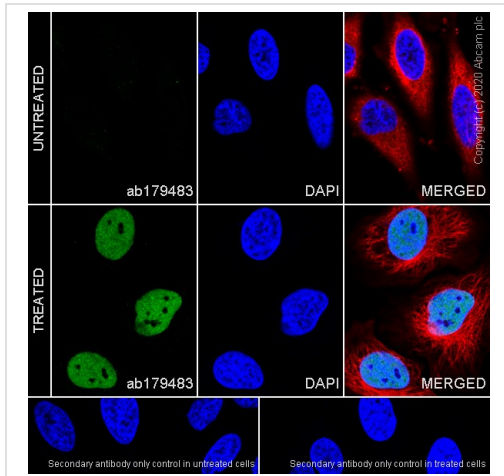
Predicted band size: 92 kDa

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.

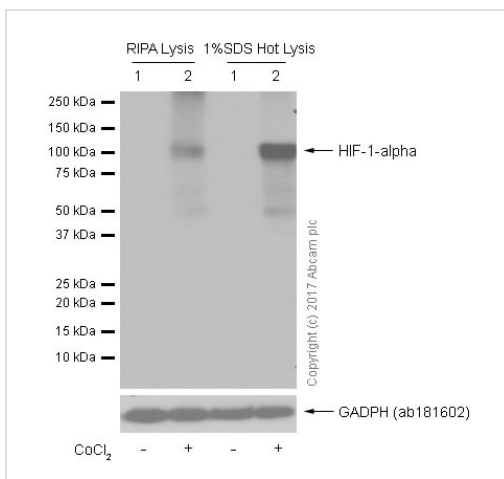
ab179483 was shown to specifically react with HIF-1 alpha in wild-

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

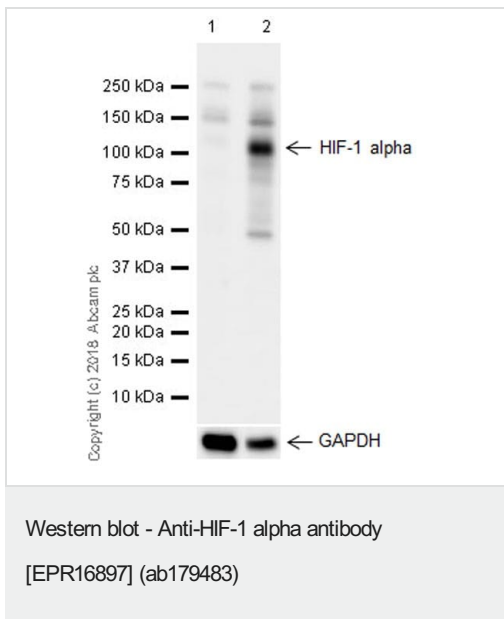
Observed band size: 110 kDa

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 \(downloadable copy\)](#).



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

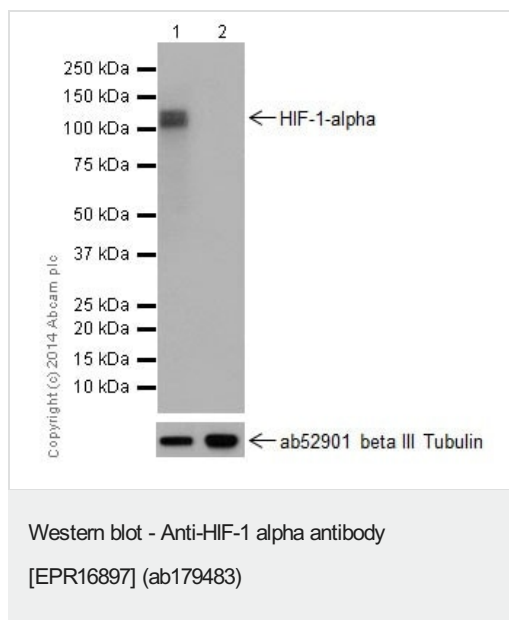
Predicted band size: 92 kDa

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).



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

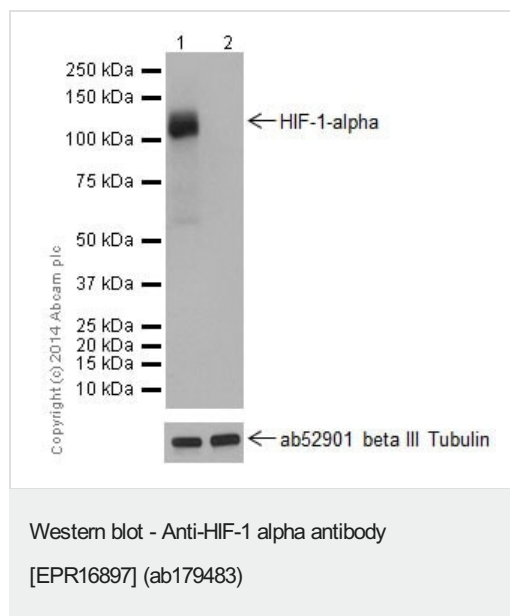
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 92 kDa

Observed band size: 110 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



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

Lane 1 : NIH/3T3 (Mouse embryo 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.

Why choose a recombinant antibody?

<p>Research with confidence Consistent and reproducible results</p>	<p>Long-term and scalable supply Recombinant technology</p>
<p>Success from the first experiment Confirmed specificity</p>	<p>Ethical standards compliant Animal-free production</p>

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

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