abcam

Product datasheet

Anti-HIF-1 alpha antibody [mgc3] ab16066

13 Abreviews 129 References 画像数 5

製品の概要

製品名	Anti-HIF-1 alpha antibody [mgc3]
製品の詳細	Mouse monoclonal [mgc3] to HIF-1 alpha
由来種	Mouse
特異性	This antibody does not cross-react with ARNT or the related HIF-2-alpha.
アプリケーション	**適用あり:** Flow Cyt, WB, IHC-P
種交差性	**交差種:** Human **交差が予測される動物種:** Mouse, Cow, Pig, Non human primates
免疫原	Recombinant fragment corresponding to Human HIF-1 alpha aa 530-826 (C terminal).
ポジティブ・コントロール	IHC-P: Human colon tissue. Human small intestine and tonsil tissue. Flow Cyt: HeLa cells. WB: HeLa wild type treated with 150uM CoCl2 for 48 hrs and HeLa Cas9 treated with 150uM CoCl2 for 48 hrs.
特記事項	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 O2 concentrations below 5% and upon stabilization under hypoxic conditions HIF-1 translocates to the nucleus. Hypoxia can be induced with treatment using certain agents e.g. $CoCl_2$ or DFO, etc. so proper sample preparation is critical. 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

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. Avoid freeze / thaw cycle.
バッファー	pH: 7.4 Preservative: 0.05% Sodium azide

	Constituent: 0.1% BSA
精製度	Protein G purified
ポリ/モノ	モノクローナル
クローン名	mgc3
アイソタイプ	IgG1

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ab16066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
Flow Cyt	★★★★★ (1)	Use 2µg for 10^6 cells. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
WB	★★★★☆ (4)	1/2000. Predicted molecular weight: 92 kDa.
IHC-P	★★★★☆ (5)	1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

ターゲット情報

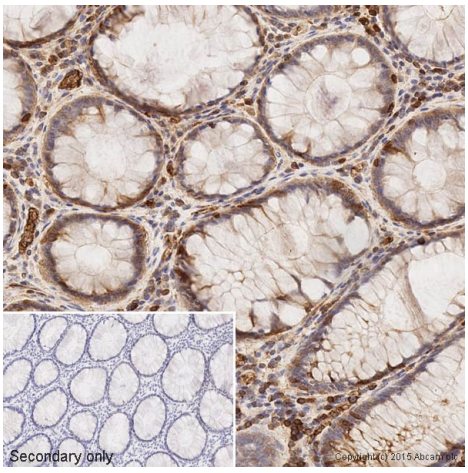
機能	Functions as a master transcriptional regulator of the adaptive response to hypoxia. Under hypoxic conditions activates the transcription of over 40 genes, including, erythropoietin, glucose transporters, glycolytic enzymes, vascular endothelial growth factor, and other genes whose protein products 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 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.

細胞内局在

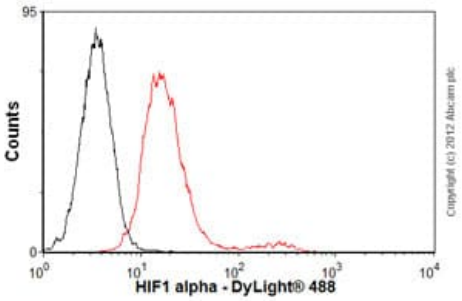
Cytoplasm. Nucleus. Cytoplasmic in normoxia, nuclear translocation in response to hypoxia. Colocalizes with SUMO1 in the nucleus, under hypoxia.

画像



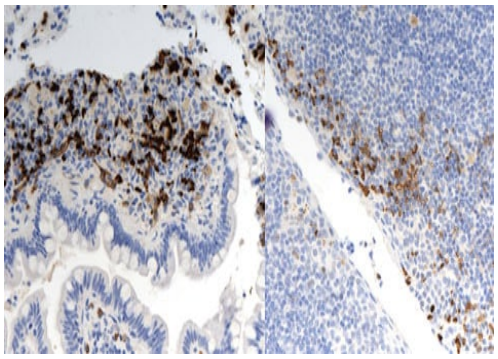
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [mgc3] (ab16066)

IHC image of ab16066 staining HIF-1 alpha in human colon formalin fixed paraffin embedded tissue sections*, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with Tris/EDTA buffer (pH9, epitope retrieval solution 2) for 20 mins. The section was then incubated with ab16066, 10µg/ml working concentration, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. No primary antibody was used in the secondary only control (shown on the inset).
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.
*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Flow Cytometry - Anti-HIF-1 alpha antibody [mgc3] (ab16066)

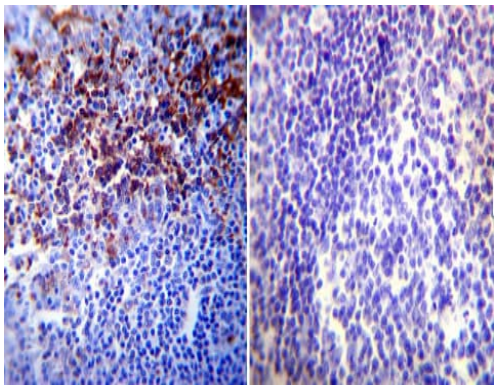
Overlay histogram showing HeLa cells stained with ab16066 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab16066, 2µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [mgc3] (ab16066)

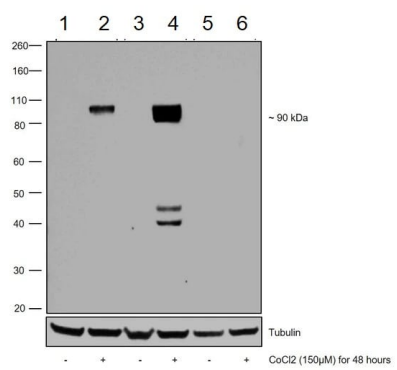
This image is courtesy of an anonymous Abreview

ab16066 staining HIF-1-alpha in Human small intestine (IBD) and tonsil tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde; antigen retrieval was by heat mediation with an EDTA buffer (pH 9.0). Samples were incubated with primary antibody (1/800 in diluent + background reducers) for 20 minutess at 25°C. An undiluted Goat polymer was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [mgc3] (ab16066)

Immunohistochemistry was performed on normal biopsies of deparaffinized Human tonsil tissue. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1/20 with ab16066 (left) or without primary antibody (negative control - right) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



Western blot - Anti-HIF-1 alpha antibody [mgc3] (ab16066)

All lanes : Anti-HIF-1 alpha antibody [mgc3] (ab16066) at 1/2000 dilution

Lane 1 : HeLa wild type (untreated)
Lane 2 : HeLa wild type treated with 150uM CoCl2 for 48 hrs
Lane 3 : HeLa Cas9
Lane 4 : HeLa Cas9 treated with 150uM CoCl2 for 48 hrs
Lane 5 : HeLa HIF1A KO
Lane 6 : HeLa HIF1A KO treated with 150uM CoCl2 for 48 hrs

Lysates/proteins at 30 µg per lane.

Secondary
All lanes : Goat anti-Mouse IgG (H+L) Superclonal™ Recombinant Secondary Antibody, HRP at 1/4000 dilution

Predicted band size: 92 kDa
Observed band size: 90 kDa

Antibody specificity was demonstrated by CRISPR-Cas9 mediated knockout of target protein. A loss of signal was observed for target protein in HIF-1 alpha KO cell line compared to control cell lines using ab16066.

Uncharacterized bands were observed in HeLa Cas9 samples at ~40 kDa and 45 kDa.

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