**Product datasheet**

**Anti-HIF-1 alpha antibody [mgc3] ab16066**

★★★★☆ 12 Abreviews   64 References   10 Images

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**Overview**

**Product name**  
Anti-HIF-1 alpha antibody [mgc3]

**Description**  
Mouse monoclonal [mgc3] to HIF-1 alpha

**Host species**  
Mouse

**Specificity**  
This antibody does not cross-react with ARNT or the related HIF-2-alpha.

**Tested applications**  
Suitable for: Flow Cyt, Immunoelectrophoresis, IHC-P, EMSA, GSA, ICC/IF, IP, WB, IHC-P

**Species reactivity**  
Reacts with: Mouse, Cow, Human, Pig, Non human primates

**Immunogen**  
Recombinant fragment corresponding to Human HIF-1 alpha aa 530-826 (C terminal).

**Positive control**  

**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 the English version and here to download the Mandarin.

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. 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.

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**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  
Constituent: 0.1% BSA

**Purity**  
Protein G purified

**Clonality**  
Monoclonal

**Clone number**  
mgc3
Isotype

IgG1

Applications

Our Abpromise guarantee covers the use of ab16066 in the following tested applications.

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

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Cyt</td>
<td>4/5</td>
<td>Use 2µg for 10^6 cells. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>Immunoelectrophoresis</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>Use a concentration of 10 µg/ml.</td>
</tr>
<tr>
<td>EMSA</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 21193390</td>
</tr>
<tr>
<td>EMSA</td>
<td></td>
<td>Use at an assay dependent concentration. Only been successfully used with mouse HIF1 alpha.</td>
</tr>
<tr>
<td>GSA</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>4/5</td>
<td>1/200.</td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>WB</td>
<td>4/5</td>
<td>1/2000. Detects a band of approximately 116 kDa (predicted molecular weight: 93 kDa). 116 kDa band represents HIF-1 alpha after hypoxic induction.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>4/5</td>
<td>1/800. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See Abreview for further information</td>
</tr>
</tbody>
</table>

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 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.

**Cellular localization**


**Images**

Western blot - Anti-HIF-1 alpha antibody [mgc3] (ab16066) at 1/1000 dilution

**All lanes**: Anti-HIF-1 alpha antibody [mgc3] (ab16066) at 1/1000 dilution

**All lanes**: Human T lymphocyte whole cell lysate

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes**: HRP-conjugated anti-mouse IgG polyclonal at 1/2000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size**: 93 kDa

**Observed band size**: 116 kDa

*why is the actual band size different from the predicted?*
Exposure time: 5 minutes

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

Immunocytochemistry/Immunofluorescence analysis of U251 cells labeling HIF-1-alpha with ab16066. HIF-1-alpha (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control - right) or with ab16066 (left) at a dilution of 1/20 over night at 4°C and incubated with a DyLight-488 conjugated secondary antibody. Images were taken at 60X magnification.

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/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2μg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed.
Immunocytochemistry/Immunofluorescence analysis of HeLa cells labeling HIF-1-alpha with ab16066. HIF-1 alpha (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control - right) or with ab16066 (left) at a dilution of 1/20 over night at 4°C and incubated with a DyLight-488 conjugated secondary antibody. Images were taken at 60X magnification.

Immunocytochemistry/Immunofluorescent analysis of HIF-1-alpha (green) in HeLa cells either left untreated (left panel) or treated with 100uM Deferoxamine mesylate for ~16 hours (right panel). Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 15 minutes at room temperature and blocked with 0.3% BSA for 15 minutes at room temperature. Cells were probed with ab16066 at a dilution of 1/100 for at least 1 hour at room temperature and washed with PBS. Cells were then incubated with a DyLight 488-conjugated goat anti-mouse IgG secondary antibody at a dilution of 1/500 for 30 minutes at room temperature. F-actin (red) was stained with DyLight 594 Phalloidin and nuclei (blue) were stained with Hoechst dye.

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 minutes at 25°C. An undiluted Goat polymer was used as the secondary antibody.
Immunocytochemistry/Immunofluorescence analysis of A2058 cells labeling HIF-1-alpha with ab16066. HIF-1-alpha (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control - right) or with ab16066 (left) at a dilution of 1/20 over night at 4°C and incubated with a DyLight-488 conjugated secondary antibody. Images were taken at 60X magnification.

Immunocytochemistry/Immunofluorescence analysis of Human fibroblasts labeling HIF-1-alpha (green) with ab16066. Cells were fixed with paraformaldehyde, permeabilized with 0.3% Triton X-100 and blocked with 5% serum for 1 hour at 24°C. Samples were incubated with primary antibody (1/500 in 0.3% Triton X-100 + 1% BSA) for 1 hour 30 minutes at 24°C. An Alexa Fluor® 488-conjugated goat anti-mouse polyclonal IgG (1/2000) was used as the secondary antibody.

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.

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