**Overview**

**Product name**  
Anti-HIF-1 alpha antibody [EP1215Y] ab51608  

**Description**  
Rabbit monoclonal [EP1215Y] to HIF-1 alpha  

**Host species**  
Rabbit  

**Specificity**  
ab51608 recognizes HIF-1-alpha. For mouse specific Hif-1-alpha rabbit monoclonal antibody, please see ab179483 (clone ID: EPR16897). ab179483 has been confirmed for mouse samples in WB.  

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

**Species reactivity**  
Reacts with: Human  

**Immunogen**  
within Human HIF-1 alpha aa 600-700 (C terminal). The exact sequence is proprietary. Database link: Q16665  
(Peptide available as ab205542)  

**Positive control**  
WB: DFO treated HeLa nuclear lysate (ab180880) and Ramos cell lysate treated with Cocl2.  
IHC-P: Human ovarian carcinoma, breast carcinoma, colonic adenocarcinoma and squamous cell cervical carcinoma tissues.  

**General notes**  
For Mouse specific Hif-1-alpha rabbit monoclonal antibody, please see ab179483 (clone ID: EPR16897).  

ab179483 has been confirmed for Mouse sample in WB.  

We have mixed customer feedback towards the rat specificity so we are unable to confirm and guarantee its performance with rat samples. Please contact technical team for more information.  

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 it.  

Our RabMAB® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAB® patents  
This product is a recombinant rabbit monoclonal antibody.
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.

Dissociation constant ($K_D$)  $K_D = 2.24 \times 10^{-10}$ M

Storage buffer  pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity  Protein A purified

Clonality  Monoclonal

Clone number  EP1215Y

Isotype  IgG

Applications

Our Abpromise guarantee covers the use of ab51608 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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<td>ICC/IF</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 20846491</td>
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| Flow Cyt    | 1/11709.   | For unpurified use at 1/15.  
ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. |
| IP          |           | Use a concentration of 5 µg/ml. |
| WB          | ⭐⭐⭐⭐⭐   | 1/100 - 1/1000. Predicted molecular weight: 93 kDa. Can be blocked with HIF-1 alpha peptide (ab205542).  
The antibody only works in hypoxic cell and tissue lysates.  
For Mouse specific Hif-1-alpha rabbit monoclonal antibody, please see ab179483 (clone ID: EPR16897).  
ab179483 has been confirmed for mouse samples in WB. |
| IHC-P       | 1/100.    | Perform heat mediated antigen retrieval via the microwave method before commencing with IHC staining protocol.  
For unpurified use at 1/15.  
For IHC antigen retrieval – See protocols IHC Antigen Retrieval Protocol. |

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

ab51608 staining HIF-1-alpha in HeLa cell line treated with Cocl2 by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody (1/500). An Alexa Fluor® 488-conjugated Goat anti-rabbit IgG(1/200) was used as the secondary antibody. Nuclei were counterstained with DAPI (right hand image).

ab51608 staining HIF-1-alpha in Human ovarian carcinoma tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed and paraffin-embedded, antigen retrieval was by heat mediation in Tris/EDTA buffer pH9. Samples were incubated with primary antibody (1/100). An undiluted HRP-conjugated anti-rabbit IgG was used as the secondary antibody. Tissue counterstained with Hematoxylin.
Western blot - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

This image is courtesy of an anonymous Abreview

**All lanes**: Anti-HIF-1 alpha antibody [EP1215Y] (ab51608) at 1/2000 dilution

**Lane 1**: MCF-7 (normoxia)
**Lane 2**: MCF-7 treated with 0.5% oxygen for 24 hours

Lysates/proteins at 30000 cells per lane.

**Secondary**

**All lanes**: Polyclonal Swine anti-rabbit IgG HRP at 1/1000 dilution

**Predicted band size**: 93 kDa

Blocking buffer: 5% milk for 16 hours at 4°C.

Anti-HIF-1 alpha antibody [EP1215Y] (ab51608) at 1/100 dilution + Ramos Cells treated with Cocl2 at 10 µg

**Secondary**

Goat Anti-Rabbit IgG, (H+L), HRP- conjugated at 1/1000 dilution

**Predicted band size**: 93 kDa
Overlay histogram showing HeLa untreated (Blue line) and HeLa treated (Red line - Deferoxamine, 1mM, 24 hours) cells stained with ab51608. 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 (ab51608, 1/11709 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&amp;L) (ab150081) at 1/2000 dilution for 30 min at 22°C.

Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.
HIF-1-alpha was immunoprecipitated using 0.5mg HeLa Nuclear DFO treated whole cell extract (ab180880), 5µg of Rabbit polyclonal to HIF1 alpha and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, HeLa DFO treated whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with unpurified ab51608.


Band: 110kDa; HIF1 alpha

HeLa cells were untreated or treated with 1mM Deferoxamine (DFO) for 24h and fixed with paraformaldehyde for imaging by fluorescent microscopy. Cells were blocked and stained with 1X blocking buffer (ab126587). Unpurified ab51608 was used at 1:500. DAPI was used to label the nucleus. HIF1 alpha staining is absent in untreated cells and induced by DFO treatment. HIF1 alpha localizes to the nucleus.
Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

Unpurified ab51608 staining HIF-1-alpha in HepG2 cells treated with baicalein (ab120723), by ICC/IF. Increase in HIF-1-alpha expression correlates with increased concentration of baicalein as described in literature.

The cells were incubated at 37°C for 6h in media containing different concentrations of ab120723 (baicalein) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature.

Staining of the treated cells with ab51608 (5 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 goat anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody.

ab51608 staining of HIF-1-alpha in untreated HeLa cell line by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde permeabilized with 0.1% Triton X-100.

Samples were incubated with primary antibody (1/500). An Alexa Fluor® 488-conjugated Goat anti-rabbit IgG(1/200) was used as the secondary antibody. Nuclei were counterstained with DAPI(right hand Image).
Immunohistochemical analysis using unpurified ab51608 showing positive staining in Breast carcinoma tissue.

Immunohistochemical analysis using unpurified ab51608 showing positive staining in Colonic adenocarcinoma tissue.

Immunohistochemical analysis using unpurified ab51608 showing positive staining in Squamous cell cervical carcinoma tissue.
**All lanes**: Anti-HIF-1 alpha antibody [EP1215Y] (ab51608) at 1/2000 dilution (Unpurified)

**Lane 1**: HeLa nuclear extract lysate (ab150036)

**Lane 2**: Hela-DFO treated (0.5mM, 24h) Nuclear Lysate (ab180880)

Lysates/proteins at 40 µg per lane.

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size**: 93 kDa

**Observed band size**: 110 kDa

**Exposure time**: 8 minutes

Abcam recommends using 5% milk as the blocking agent, decreasing to 2% milk during primary and secondary incubation. Abcam welcomes customer feedback and would appreciate any comments regarding this product and the data presented above.
Anti-HIF-1-alpha unpurified antibody (ab51608) reactivity with reduced Hep3B cell lysate after transient transfection of scrambled siRNA (lanes 1-3 and 7-9) or HIF-1-alpha siRNA (lanes 4-6 and 10-12). Cells were incubated at with 21% O\textsubscript{2} (lanes 1-6) or 1% O\textsubscript{2} (lanes 7-12) for 4h before lysis. After SDS-PAGE, membranes were blocked in 5% milk for 1h at 25°C before incubation with unpurified ab51608 (1/1,000 dilution 5% milk) for 16h at 4°C. The blot was then incubated with an anti-Rabbit HRP-conjugated secondary antibody before developing with ECL.
Western blot - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

All lanes: Anti-HIF-1 alpha antibody [EP1215Y] (ab51608) at 1/2000 dilution (unpurified)

Lane 1: HeLa Whole Cell Lysate (untreated, negative control)
Lane 2: HeLa DFO treated (0.5mM, 24h) Whole Cell Lysate
Lane 3: HeLa Nuclear Cell Lysate (untreated, negative control)
Lane 4: HeLa Nuclear DFO treated (0.5mM, 24h) Cell Lysate

Lysates/proteins at 40 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 93 kDa
Observed band size: 110 kDa

Exposure time: 2 minutes

Abcam recommends using 5% milk as the blocking agent, decreasing to 2% milk during primary and secondary incubation. Abcam welcomes customer feedback and would appreciate any comments regarding this product and the data presented above.
Other - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

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