abcam

Product datasheet

Anti-HIF-1 alpha antibody [EP1215Y] ab51608

重组 RabMAb

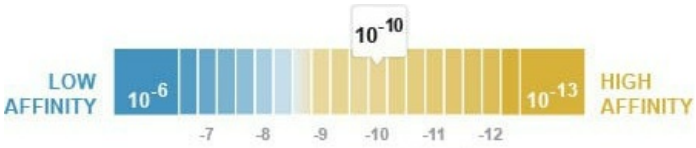
15 Abreviews 304 References 21 图像

概述

产品名称	Anti-HIF-1 alpha抗体[EP1215Y]
描述	兔单克隆抗体[EP1215Y] to HIF-1 alpha
宿主	Rabbit
特异性	This antibody 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.
经测试应用	**适用于:** ICC/IF, ChIC/CUT&RUN-seq, Flow Cyt (Intra), IP, WB, IHC-P
种属反应性	**与反应:** Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: DFO treated HeLa nuclear lysate (**ab180880**), Ramos cell lysate treated with Cocl2. IHC-P: Human ovarian and breast carcinoma, colonic adenocarcinoma and squamous cell cervical carcinoma tissues. Human gastric cancer and CRC tumour tissue ICC/IF: DFO treated Hela cells, Cocl2 treated HeLa cells and baicalein treated HepG2 cells Flow Cyt (intra): DFO treated HeLa cells IP: DFO treated HeLa nuclear lysate ChIC/CUT&RUN-Seq: HeLa cells.
常规说明	**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. 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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
解离常数 (K_D)	K_D = 2.24 x 10 $^{-10}$ M



Learn more about K_D

存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EP1215Y
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab51608于以下的经测试应用

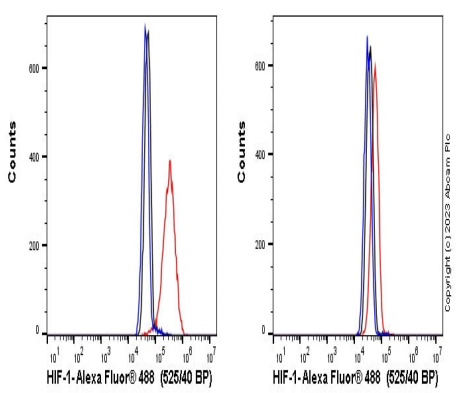
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF		1/500.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5 µg
Flow Cyt (Intra)		1/10000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP		Use a concentration of 5 µg/ml.
WB	★★★★☆ (10)	1/100 - 1/1000. Predicted molecular weight: 93 kDa. 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	★★★★★ (3)	1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. For IHC antigen retrieval – See protocols **IHC Antigen Retrieval Protocol**.

靶标

功能	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.
组织特异性	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.
序列相似性	Contains 1 basic helix-loop-helix (bHLH) domain. Contains 1 PAC (PAS-associated C-terminal) domain. Contains 2 PAS (PER-ARNT-SIM) domains.
结构域	Contains two independent C-terminal transactivation domains, NTAD and CTAD, which function synergistically. Their transcriptional activity is repressed by an intervening inhibitory domain (ID).
翻译后修饰	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.
细胞定位	Cytoplasm. Nucleus. Cytoplasmic in normoxia, nuclear translocation in response to hypoxia. Colocalizes with SUMO1 in the nucleus, under hypoxia.

图片



Flow Cytometry (Intracellular) - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

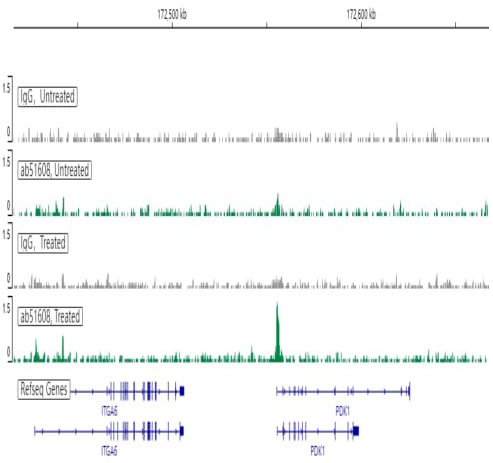
Flow cytometry overlay histogram showing left, HeLa treated with 1mM Deferoxamine for 24h and right, negative untreated HeLa stained with ab51608 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab51608) (1x 10^6 in 100µl at 0.2µg/ml (1/11000)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody gave a positive signal in HeLa Fixed with 4% formaldehyde (10 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.

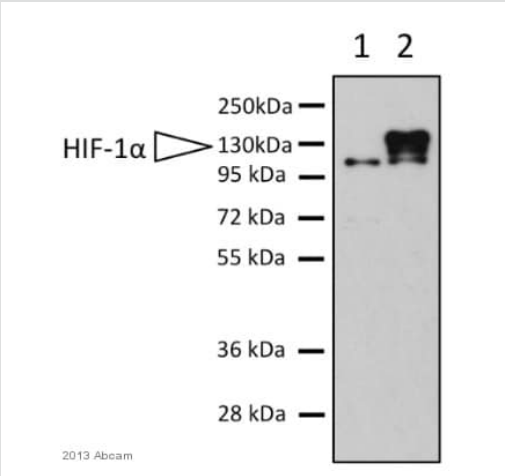


ChIC/CUT&RUN sequencing - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

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 ab51608 [EP1215Y]. 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.



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

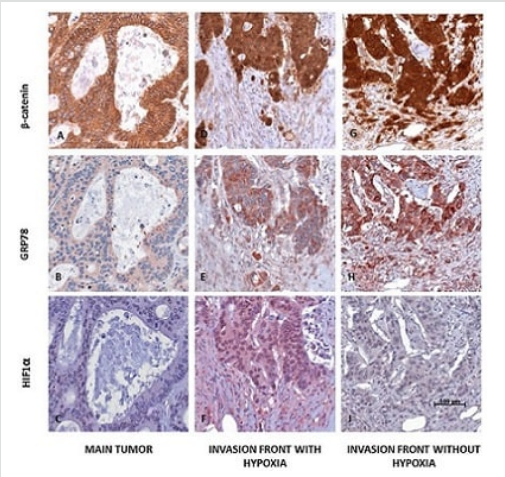
Lane 1 : MCF-7 (normoxia)
Lane 2 : MCF-7 treated with 0.5% oxygen for 24 hours

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.

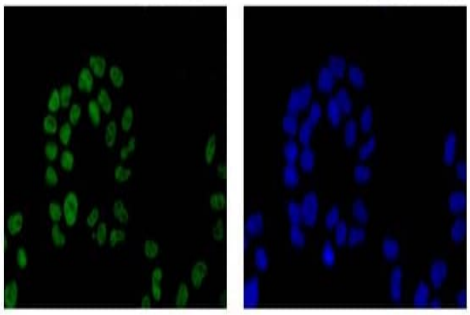


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

Zeindl-Eberhart E et al. Epithelial-mesenchymal transition induces endoplasmic-reticulum-stress response in human colorectal tumor cells. PLoS One 9:e87386 (2014).

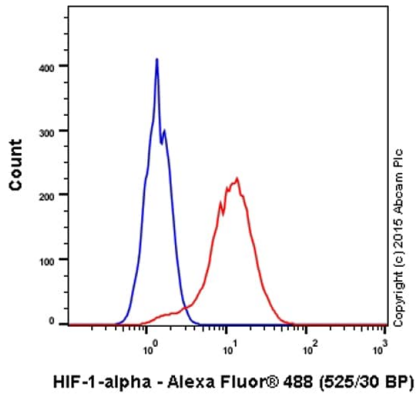
Immunohistochemical analysis of Formalin-fixed paraffin-embedded human CRC tumour tissue using ab51608 for HIF-1 alpha staining. Endogenous peroxidase of sections was inhibited by 7.5% H_2O_2 at room temperature

In central tumor areas of human CRCs β-catenin was typically localized at the cell membrane (A) whereas only a weak staining was observed for cytoplasmic GRP78 (B) and **HIF-1 alpha** staining was found to be negative (C). At the invasion front strong nuclear β-catenin was detectable indicating EMT (D, G). In corresponding regions strong cytoplasmic GRP78 expression was found (E, H). In some of the cases an intense nuclear **HIF-1 alpha** staining was observed (F, with hypoxia), but not in others (I, without hypoxia) (magnification 200×; scale bar: 100 µm).



Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

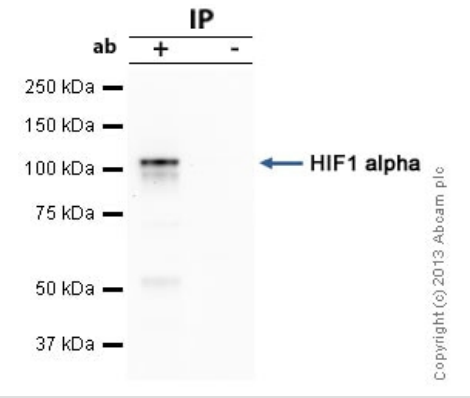
ab51608 staining HIF-1-alpha in HeLa cell line treated with Cocl2 by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody (1/500). An Alexa Fluor® 488-conjugated Goat anti-rabbit IgG(1/200)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



Flow Cytometry (Intracellular) - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

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 Fluorr®488 goat anti-rabbit IgG (H&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.



Immunoprecipitation - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

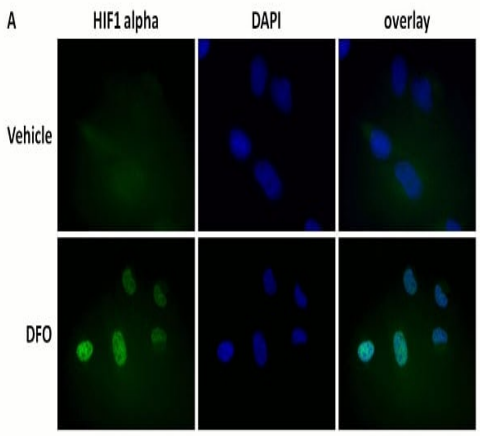
HIF-1-alpha was immunoprecipitated using 0.5mg HeLa Nuclear DFO treated whole cell extract (**ab180880**), 5µg of Rabbit polyclonal to HIF1 alpha and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

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.

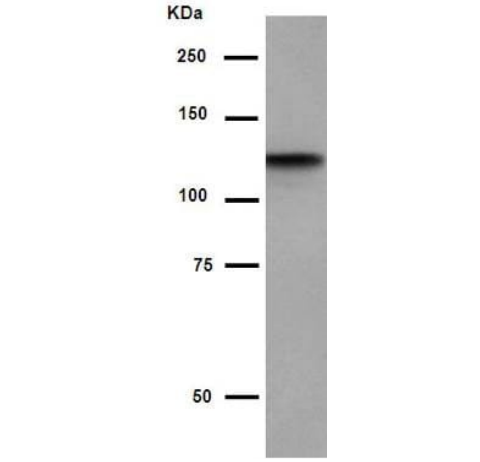
Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (**ab99697**).

Band: 110kDa; HIF1 alpha



Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

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.



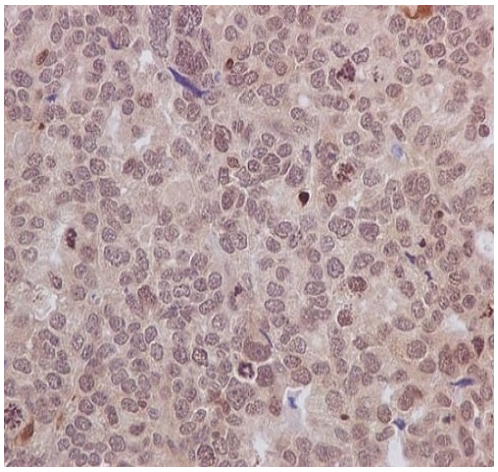
Western blot - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

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

Secondary

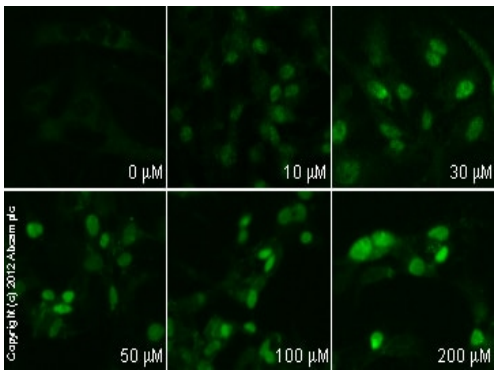
Goat Anti-Rabbit IgG, (H+L), HRP- conjugated at 1/1000 dilution

Predicted band size: 93 kDa



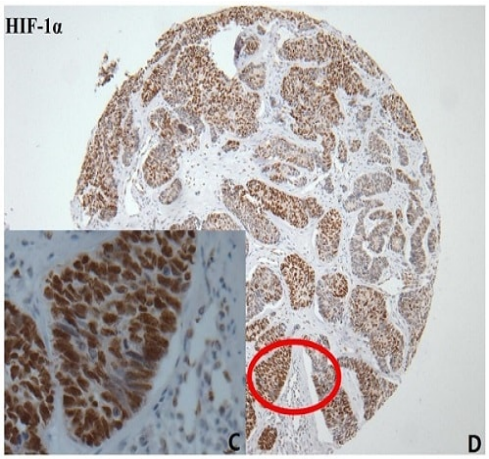
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

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.



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.

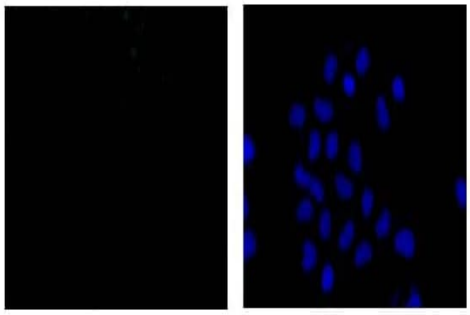


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

Chen L et al. HIF-1 alpha overexpression correlates with poor overall survival and disease-free survival in gastric cancer patients post-gastrectomy. PLoS One 9:e90678 (2014).

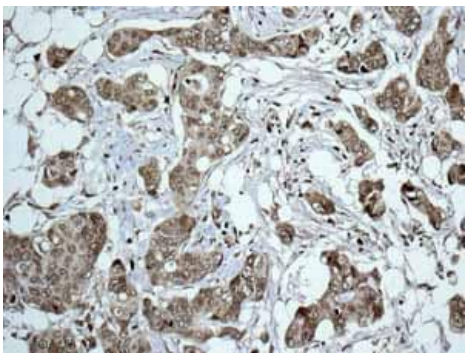
Immunohistochemical analysis of paraffin-embedded formalin-fixed human gastric cancer tissue stained for HIF-1 alpha using **ab15608** at 1/600 dilution. Tissue sections were counterstained with Mayer's hematoxylin. Citrate buffer (pH 6.0) antigen retrieval using standard methodology

C. HIF-1 alpha was located mainly in the nucleus of tumor cells (positive expression ×400).
D. HIF-1 alpha original magnification ×100.



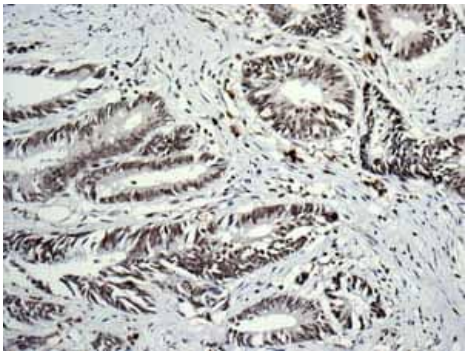
Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

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).



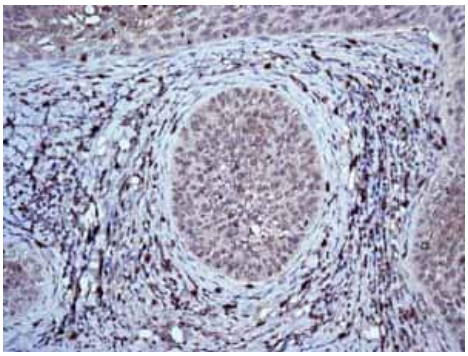
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

Immunohistochemical analysis using unpurified ab51608 showing positive staining in Breast carcinoma tissue.



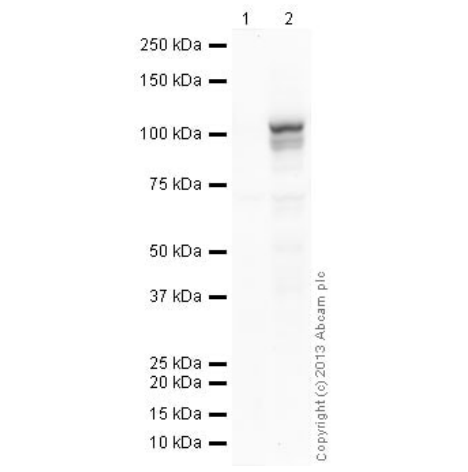
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

Immunohistochemical analysis using unpurified ab51608 showing positive staining in Colonic adenocarcinoma tissue. Heat mediated antigen retrieval was performed via the microwave method before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

Immunohistochemical analysis using unpurified ab51608 showing positive staining in Squamous cell cervical carcinoma tissue. Heat mediated antigen retrieval was performed via the microwave method before commencing with IHC staining protocol.



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 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

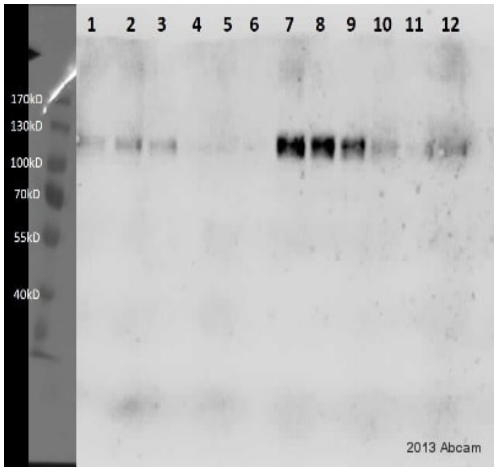
Developed using the ECL technique.

Performed under reducing conditions.

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

Exposure time: 8 minutes

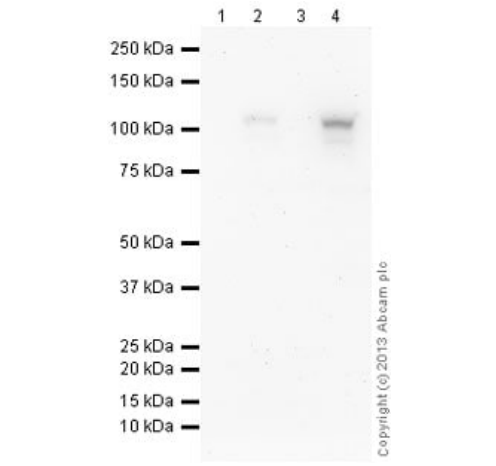
Abcam recommends using 5% milk as the blocking agent, decreasing to 2% milk during primary and secondary incubation. Abcam welcomes customer feedback and would appreciate any comments regarding this product and the data presented above.



Western blot - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

Image courtesy of Teresa Otto (University of Duisburg-Essen, Germany)

Anti-HIF-1-alpha unpurified antibody (ab51608) reactivity with reduced Hep3B cell lysate after transient transfection of scrambled siRNA (lanes1-3 and 7-9) or HIF-1-alpha siRNA (lanes 4-6 and 10-12). Cells were incubated at with 21% O_2 (lanes 1-6) or 1% O_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 4ºC. 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

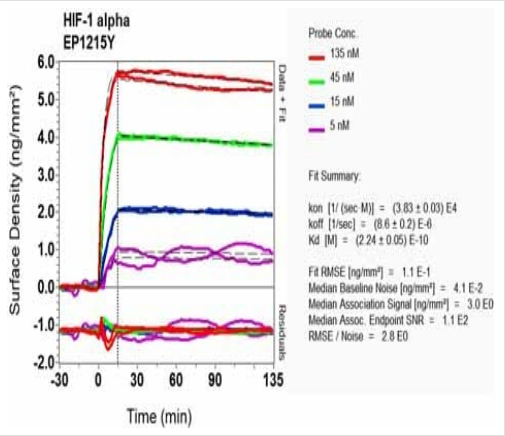
Performed under reducing conditions.

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.



OI-RD Scanning - 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

Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

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