abcam

Product datasheet

Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free ab210073

Recombinant RabMAb

1 Abreviews 21 References 19 Images

Overview

Product name	Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free
Description	Rabbit monoclonal [EP1215Y] to HIF-1 alpha - BSA and Azide free
Host species	Rabbit
Specificity	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.
Tested applications	**Suitable for:** ICC/IF, WB, ChIC/CUT&RUN-seq, Flow Cyt (Intra), IP, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	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.
General notes	ab210073 is the carrier-free version of **ab51608**. **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. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

This product is a recombinant monoclonal antibody, which offers several advantages including:

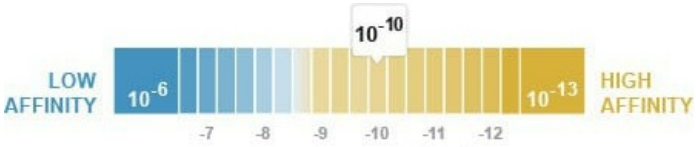
- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production

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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Dissociation constant (K_D)	$K_D = 2.24 \times 10^{-10}$ M



Learn more about K_D

Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP1215Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab210073 in the following tested applications.

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

Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 120 kDa (predicted molecular weight: 92 kDa). Please check the parent abID, **ab51608**, for more information on dilutions.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5 µg

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP		Use at an assay dependent concentration.
IHC-P	★★★☆☆ (1)	Use at an assay dependent concentration. 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 protocols**.

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

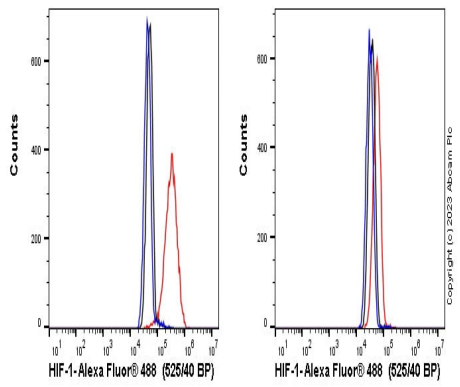
Contains two independent C-terminal transactivation domains, NTAD and CTAD, which function synergistically. Their transcriptional activity is repressed by an intervening inhibitory domain (ID).

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

Images



Flow Cytometry (Intracellular) - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab51608**).

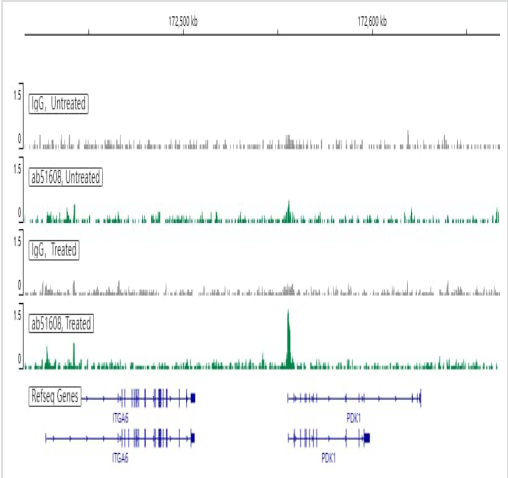
Flow cytometry overlay histogram showing left, HeLa treated with 1mM Deferoxamine for 24h and right, negative untreated HeLa stained with **ab51608** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated 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.



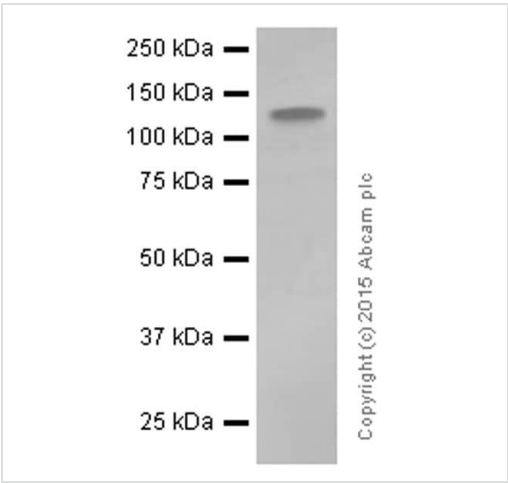
ChIC/CUT&RUN sequencing - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab51608**).



Western blot - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073) + Ramos (human Burkitt's lymphoma) treated with cocl2 whole cell lysate at 10 µg

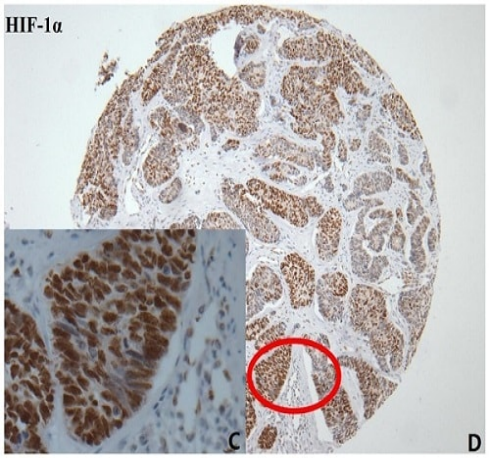
Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**)

Predicted band size: 92 kDa
Observed band size: 120 kDa

Exposure time: 3 minutes

Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM/TBST



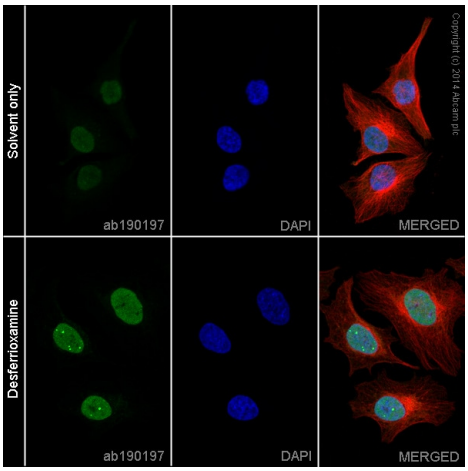
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

Image from 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab51608**).

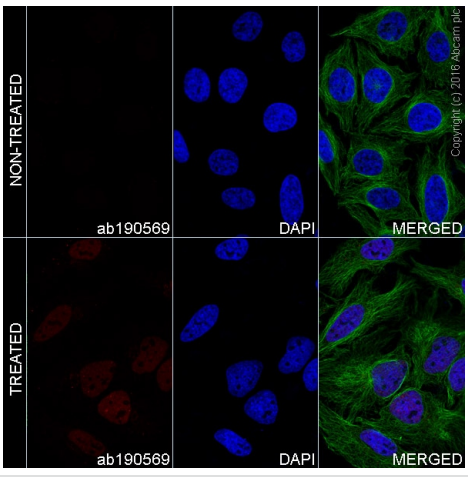


Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

Clone EP1215Y (ab210073) has been successfully conjugated by Abcam. This image was generated using Anti-HIF-1 alpha antibody [EP1215Y] (Alexa Fluor® 488). Please refer to **ab190197** for protocol details.

ab190197 staining HIF-1α in DFO-treated HeLa cells. The cells were treated with 1mM Desferrioxamine (DFO) for 24 hours or solvent-only for control purposes. The cells were fixed with 4% formaldehyde (10 min), permeabilized in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with **ab190197** at a working dilution of 1 in 100 (shown in green) and **ab195889**, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor® 594, shown in red) at a diltuion of 1 in 250 overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



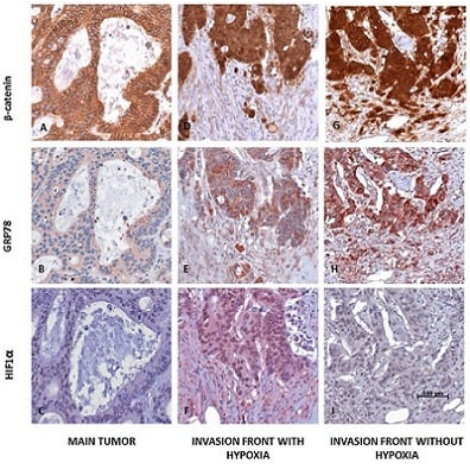
Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

Clone EP1215Y (ab210073) has been successfully conjugated by Abcam. This image was generated using Anti-HIF-1 alpha antibody [EP1215Y] (Alexa Fluor® 647). Please refer to **ab190569** for protocol details.

ab190569 staining HIF-1-alpha in HeLa cells +/- $CoCl_2$ (0.5mM, 16 hours). The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h.

The cells were then incubated overnight at +4°C with **ab190569** at 1/50 dilution (shown in red) and **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



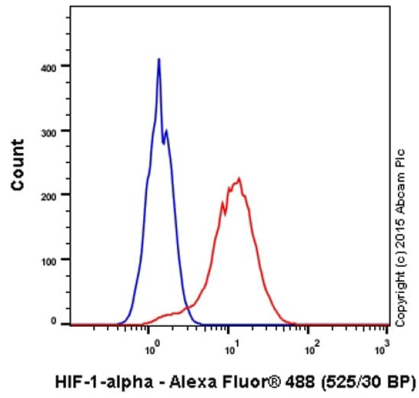
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

Image from 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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab51608**).

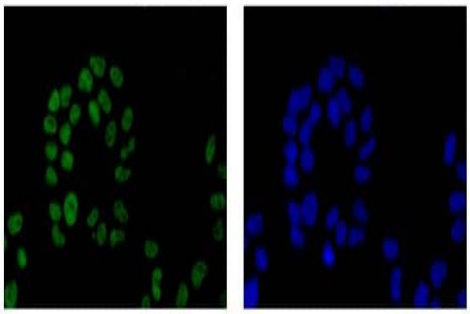


Flow Cytometry (Intracellular) - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

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&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.

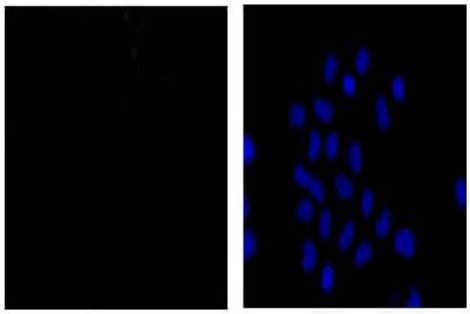
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab51608**).



Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

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).

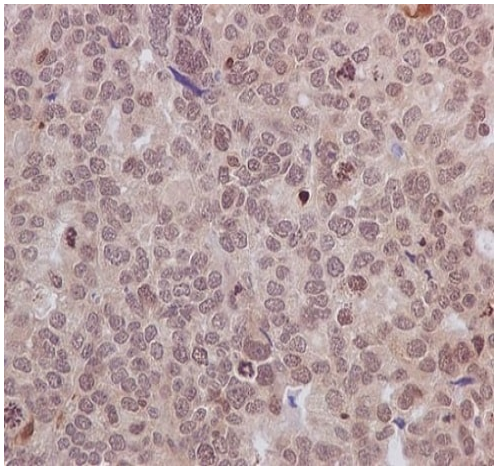
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab51608**).



Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

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).

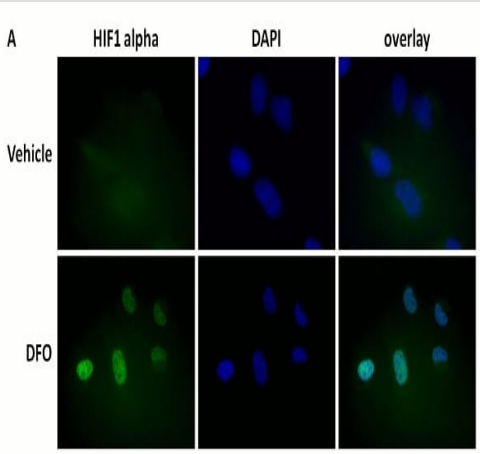
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab51608**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

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.

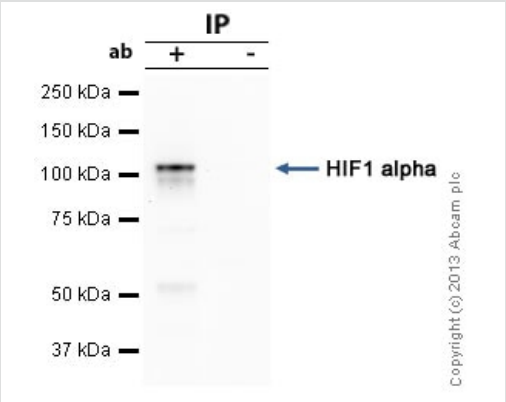
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab51608**).



Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab51608**).



Immunoprecipitation - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

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 (-).

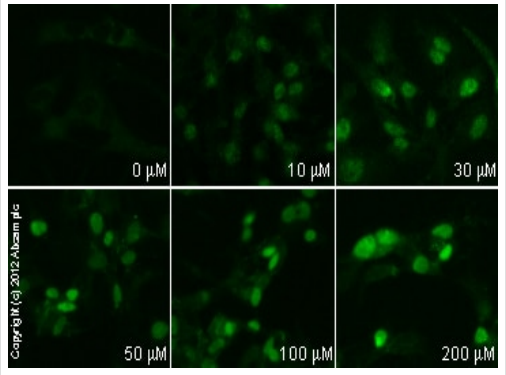
The antibody was incubated under agitation with Protein G beads for 10min, HeLa DFO treated whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

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

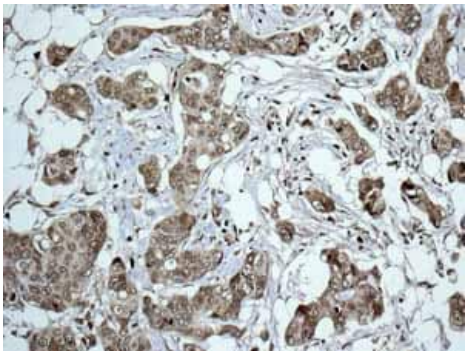
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab51608**).



Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

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.

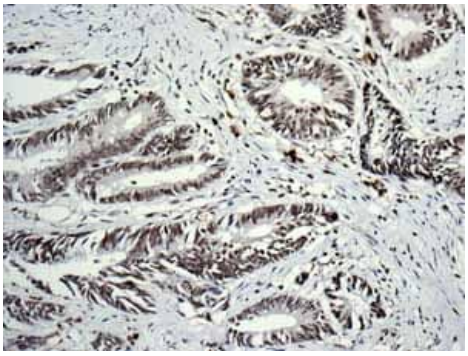
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab51608**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

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

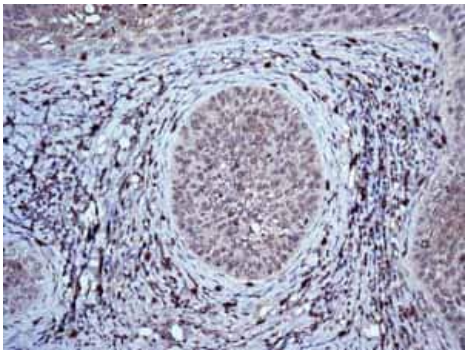
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab51608**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

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

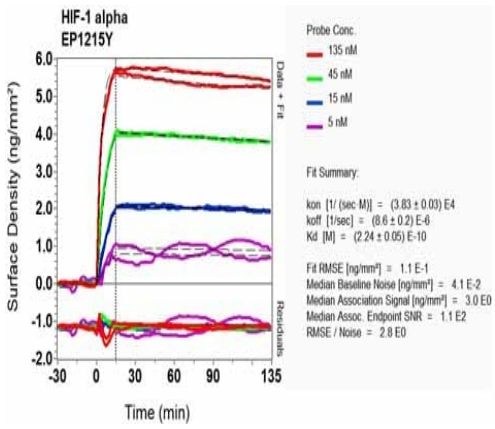
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab51608**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

Immunohistochemical analysis using unpurified **ab51608** showing positive staining in Squamous cell cervical carcinoma tissue.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab51608**).



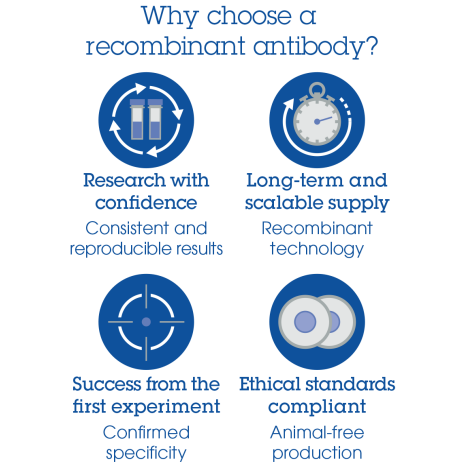
OI-RD Scanning - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab51608**).



Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

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