abcam

ab210073

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

Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073) is a rabbit recombinant monoclonal antibody provided in a PBS only buffer for easy conjugation. Suitable for **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF** in **Human**.

- KO validated for confirmed specificity
- BSA, sodium azide, and glycerol-free for easy conjugation
- Biophysical QC for unrivalled batch-batch consistency
- Over 20 publications

Recombinant | RabMAb | Advanced Validation | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EP1215Y
Purification technique	Affinity purification Protein A
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. HIF-1 alpha expression is absent in most normal tissues (PMID: 12128120, 24835245, 11689469, 20217131).
Dissociation constant	2.24 x 10^{-10} M
Concentration	1.014 mg/mL The concentration of this product may be batch-dependent

Batch concentration finder →

Reactivity data

ChIC/CUT&RUN-seq

Tested

Species	Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	Please check the parent abID, ab51608, for more information on dilutions. Compared with ab51608, ab308433 has higher sensitivity, we recommend ab308433 as an alternative for testing HIF-1 alpha in western blot.

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

IHC-P

Tested

Species	Human
Dilution info	-
Notes	For IHC antigen retrieval - See protocols .

Target data

See full target information HIF1A

Function

Functions as a master transcriptional regulator of the adaptive response to hypoxia (PubMed:11292861, PubMed:11566883, PubMed:15465032, PubMed:16973622, PubMed:17610843, PubMed:18658046, PubMed:20624928, PubMed:22009797, PubMed:30125331, PubMed:9887100). Under hypoxic conditions, activates the transcription of over 40 genes, including erythropoietin, glucose transporters, glycolytic enzymes, vascular endothelial growth factor, HILPDA, and other genes whose protein products increase oxygen delivery or facilitate metabolic adaptation to hypoxia (PubMed:11292861, PubMed:11566883, PubMed:15465032, PubMed:16973622, PubMed:17610843, PubMed:20624928, PubMed:22009797, PubMed:30125331, PubMed:9887100). Plays an essential role in embryonic vascularization, tumor angiogenesis and pathophysiology of ischemic disease (PubMed:22009797). Heterodimerizes with ARNT; heterodimer binds to core DNA sequence 5'-TACGTG-3' within the hypoxia response element (HRE) of target gene promoters (By similarity). Activation requires recruitment of transcriptional coactivators such as CREBBP and EP300 (PubMed:16543236, PubMed:9887100). Activity is enhanced by interaction with NCOA1 and/or NCOA2 (PubMed:10594042). Interaction with redox regulatory protein APEX1 seems to activate CTAD and potentiates activation by NCOA1 and CREBBP (PubMed:10202154, PubMed:10594042). Involved in the axonal distribution and transport of mitochondria in neurons during hypoxia (PubMed:19528298). (Microbial infection) Upon infection by human coronavirus SARS-CoV-2, is required for induction of glycolysis in monocytes and the consequent pro-inflammatory state (PubMed:32697943). In monocytes, induces expression of ACE2 and cytokines such as IL1B, TNF, IL6, and interferons (PubMed:32697943). Promotes human

coronavirus SARS-CoV-2 replication and monocyte inflammatory response (PubMed:32697943).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

What is this antibody validated in?
Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human samples.

What is the molecular weight of HIF-1 alpha?
Anti-HIF-1 alpha [EP1215Y] - BSA and Azide free (ab210073) specifically detects a band for HIF-1 alpha (UniProt: Q16665) at a molecular weight of 92kDa.

Trusted by the scientific community
Anti-HIF-1 alpha [EP1215Y] - BSA and Azide free (ab210073) was first used in a scientific publication in 2015 and has been cited over 20 times in peer-reviewed journals.

Specificity confirmed
The specificity of Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073) has been confirmed by Western blot testing in HIF1A Knockout HCT 116 cells.

Other related products
We have a range of other formats of antibody clone [EP1215Y] also available for your convenience: ab51608, Alexa Fluor® 647 - ab190569, Carrier free - ab210073

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	HIF-1 alpha also known as hypoxia-inducible factor 1-alpha is a transcription factor critical in cellular response to low oxygen levels. Its molecular weight usually ranges from 93 to 120 kDa. You can find HIF-1 alpha expressed in tissues throughout the body but its expression significantly increases under hypoxic conditions. Researchers often use the HIF-1a ELISA to measure its expression levels. HIF-1 alpha forms a complex with other proteins to perform its functions effectively.
Biological function summary	HIF-1 alpha regulates gene expression in response to hypoxic conditions in cells. It forms a complex with HIF-1 beta to activate transcription of various genes involved in energy metabolism angiogenesis and erythropoiesis. HIF-1 alpha enables cells to adapt to reduced oxygen availability allowing for cellular survival and function under stress. It plays an important role in promoting the expression of genes like VEGF and EPO which are important for vascular and red blood cell development respectively.
Pathways	HIF-1 alpha plays an integral role in the hypoxia signaling pathway and the glycolytic pathway. In the hypoxia signaling pathway HIF-1 alpha partners with VHL (Von Hippel-Lindau) protein that regulates its degradation under normal oxygen conditions. When oxygen levels drop HIF-1 alpha avoids degradation stabilizes and translocates into the nucleus to initiate transcription of hypoxia-responsive genes. The glycolytic pathway involvement highlights its function in adapting energy production under hypoxic conditions through collaboration with enzymes and transporters associated with glycolysis.
Associated diseases and disorders	HIF-1 alpha has been implicated in cancer and ischemic diseases. Its role in promoting angiogenesis and metabolic adaptation makes it a contributor to tumor growth and survival collaborating with oncogenes such as c-Myc. In ischemic diseases like stroke or myocardial infarction HIF-1 alpha's ability to induce protective responses can mitigate tissue damage through regulation of survival pathways. Understanding these interactions helps in the development of therapeutic strategies targeting HIF-1 alpha in disease contexts.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

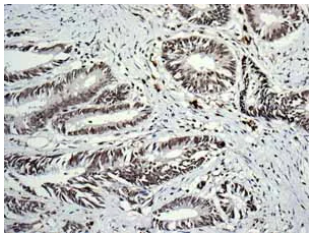
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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21 product images

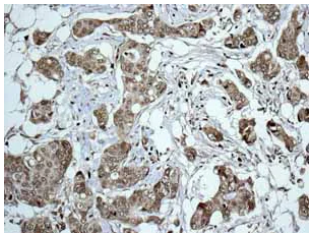


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

HIF-1 alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HIF-1 alpha antibody

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).

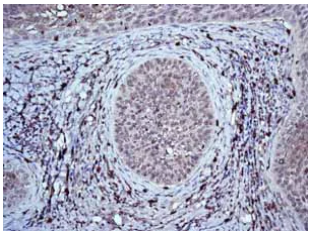


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

HIF-1 alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HIF-1 alpha antibody

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).

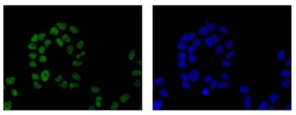


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

HIF-1 alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HIF-1 alpha antibody

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

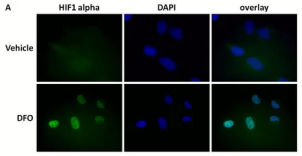
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab51608).



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

HIF-1 alpha Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HIF-1 alpha antibody

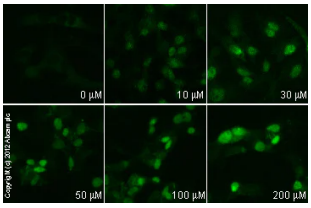
ab51608 staining HIF-1-alpha in HeLa cell line treated with Cocl2 by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody (1/500). An Alexa Fluor® 488-conjugated Goat anti-rabbit IgG(1/200)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

HIF-1 alpha Immunocytochemistry/ Immunofluorescence staining of HeLa cells using rabbit Anti-HIF-1 alpha antibody

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).



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

HIF-1 alpha Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HIF-1 alpha antibody

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).

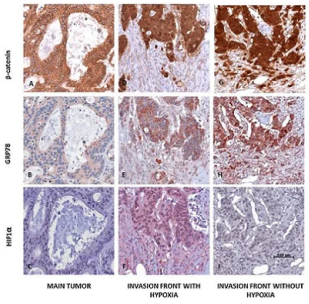


Image from Zeindl-Eberhart E et al. Epithelial-mesenchymal transition induces endoplasmic-reticulum-stress response in human colorectal tumor cells. PLoS One 9:e87386 (2014).

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

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).

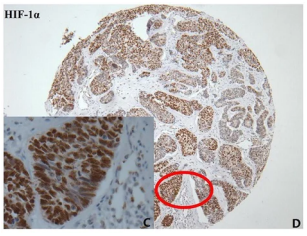
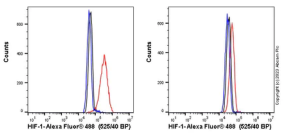


Image from Chen L et al. HIF-1 alpha overexpression correlates with poor overall survival and disease-free survival in gastric cancer patients post-gastrectomy. PLoS One 9:e90678 (2014).

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

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).



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

HIF-1 alpha Flow Cytometry (Intracellular) staining using rabbit Anti-HIF-1 alpha antibody

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

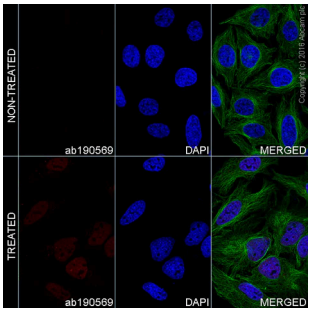
Flow cytometry overlay histogram showing left, HeLa treated with 1mM Deferoxamine for 24h and right, negative untreated HeLa stained with ab51608 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab51608) (1x 106 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.



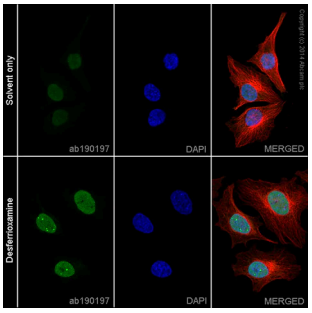
Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

HIF-1 alpha Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HIF-1 alpha antibody

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).



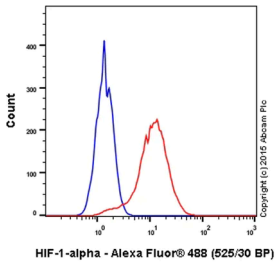
Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

HIF-1 alpha Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HIF-1 alpha antibody

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).

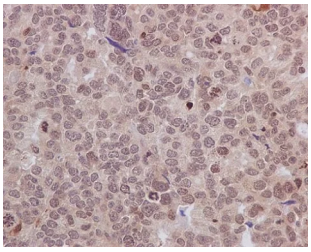


Flow Cytometry (Intracellular) - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

HIF-1 alpha Flow Cytometry (Intracellular) staining using rabbit Anti-HIF-1 alpha antibody

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.

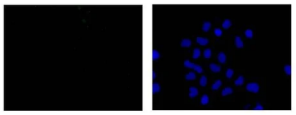
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab51608).



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

HIF-1 alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HIF-1 alpha antibody

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).

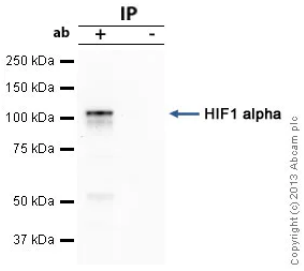


Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

HIF-1 alpha Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HIF-1 alpha 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).
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).

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

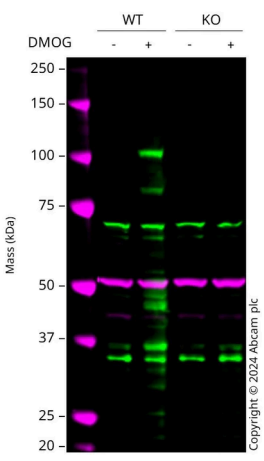
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 92 kDa

Observed band size: 110 kDa

Exposure time: 12min



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

HIF-1 alpha Western blot staining using rabbit Anti-HIF-1 alpha antibody

Western blot: Anti-HIF1A antibody [EP1215Y] (ab51608) staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab51608 was shown to bind specifically to HIF1A. A band was observed at 105 kDa in wild-type Wild-type HCT 116 DMOG (0 mM, 4 h) cell lysates with no signal observed at this size in HIF1A knockout cell line. To generate this image, wild-type and HIF1A knockout Wild-type HCT 116 DMOG (0 mM, 4 h) cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:

Western blot - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608) at 1/1000 dilution

Lane 1:
Wild-type HCT116 DMOG (0 mM, 4 h) cell lysate at 20 µg

Lane 2:
Wild-type HCT116 treated DMOG (1 mM, 4 h) cell lysate at 20 µg

Lane 3:
HIF1A knockout HCT116 DMOG (0 mM, 4 h) cell lysate at 20 µg

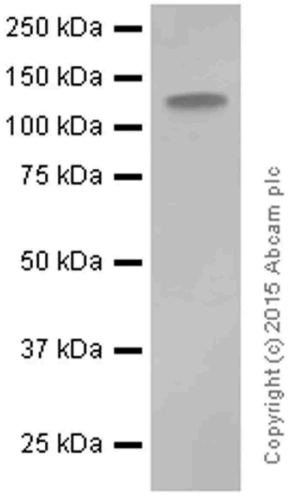
Lane 4:
HIF1A knockout HCT116 treated DMOG (1 mM, 4 h) cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 105 kDa



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

HIF-1 alpha Western blot staining of Ramos (human Burkitt's lymphoma) treated with cocl2 whole cell lysate using rabbit Anti-HIF-1 alpha antibody

Blocking buffer and concentration: 5% NFDM/TBST
Diluting buffer and concentration: 5% NFDM/TBST

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

All lanes:
Ramos (human Burkitt's lymphoma) treated with cocl2 whole cell lysate at 10 µg

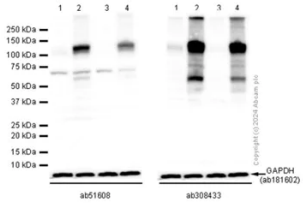
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051)

Predicted band size: 92 kDa

Observed band size: 120 kDa

Exposure time: 3min



Western blot - 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). Blocking/Diluting buffer and concentration: 5% NFDM/TBST Compared with ab51608, ab308433 has higher sensitivity, we recommend ab308433 as an alternative for testing HIF-1 alpha in western blot.
Lanes 1 - 4:
Western blot - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608)

Lanes 1 - 4:
Western blot - Anti-HIF-1 alpha antibody [EP1215Y] - BSA and Azide free (ab210073)

Lanes 1 and 3:
Untreated HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

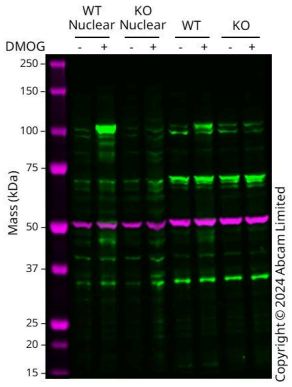
Lane 2:
HeLa treated with 0.5mM CoCl2 for 6h whole cell lysate at 20 µg

Lane 4:
HeLa treated with 0.5mM DFO for 24h whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 110 kDa



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

HIF-1 alpha Western blot staining using rabbit Anti-HIF-1 alpha antibody

This data was developed using the same antibody clone in a different buffer formulation (ab51608).

Western blot: Anti-HIF1A antibody [EP1215Y] (ab51608) staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab51608 was shown to bind specifically to HIF1A. A band was observed at 100 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in HIF1A knockout cell line. To generate this image, wild-type and HIF1A knockout HCT 116 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-HIF-1 alpha antibody [EP1215Y] (ab51608) at 1/1000 dilution

Lane 1:
Wild-type HCT 116 DMOG (0 mM, 4 h) nuclear cell lysate at 20 µg

Lane 2:
Wild-type HCT 116 treated DMOG (1 mM, 4 h) nuclear cell lysate at 20 µg

Lane 3:
HIF1A knockout HCT 116 DMOG (0 mM, 4 h) nuclear cell lysate at 20 µg

Lane 4:
HIF1A knockout HCT 116 treated DMOG (1 mM, 4 h) nuclear cell lysate at 20 µg

Lane 5:
Wild-type HCT 116 DMOG (0 mM, 4 h) cell lysate at 20 µg

Lane 6:
Wild-type HCT 116 treated DMOG (1 mM, 4 h) cell lysate at 20 µg

Lane 7:
HIF1A knockout HCT 116 DMOG (0 mM, 4 h) cell lysate at 20 µg

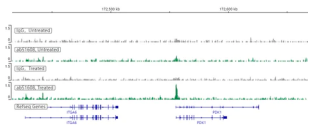
Lane 8:
HIF1A knockout HCT 116 treated DMOG (1 mM, 4 h) cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

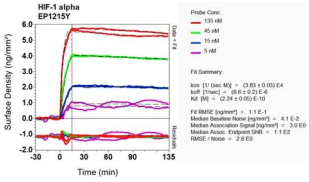
Performed under reducing conditions.

Observed band size: 100 kDa



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 found in the Protocol booklet in the Product Protocol section. 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).



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

We have systematically measured KD (the equilibrium dissociation constant between the antibody and its antigen), of more than 840 recombinant antibodies to assess not only their individual KD values but also to see the average affinity of antibody. Based on the comparison with published literature values for mouse monoclonal antibodies, Recombinant antibodies appear to be on average 1-2 order of magnitude higher affinity.

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