abcam

ab308433

Anti-HIF-1 alpha antibody [EPR16897-145]

Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433) is a rabbit monoclonal antibody detecting HIF-1 alpha in **Western Blot, Flow Cytometry (Intra), IP, ICC/IF, ChIP**. Suitable for **Human, Mouse**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency

Recombinant | RabMAb | Advanced Validation | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR16897-145
Purification technique	Affinity purification Protein A
Specificity	Unsuitable for mouse IP. HIF-1 alpha expression is absent in most normal tissues (PMID: 12128120, 24835245, 11689469, 20217131).
Concentration	0.495 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ChIP-seq

Tested

Species	Human
Dilution info	8 µg for 10^7 Cells
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

ChIP

Tested

Species	Human
Dilution info	5 µg for 25 µg chromatin
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Rat
Dilution info	-

Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	We recommend using a high-sensitivity ECL substrate capable of detecting proteins in the mid-femtogram range when testing this antibody by Western blot, to ensure optimal signal detection.

Species	Mouse
Dilution info	1/1000
Notes	We recommend using a high-sensitivity ECL substrate capable of detecting proteins in the mid-femtogram range when testing this antibody by Western blot, to ensure optimal signal detection.

Not recommended

Species	Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100
Notes	-

Species	Mouse
Dilution info	1/100
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/50
Notes	-

Species	Mouse
Dilution info	1/50
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

ChIC/CUT&RUN-seq

Tested

Species	Human
Dilution info	5 µg
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

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	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunocytochemistry/immunofluorescence (ICC/IF), ChIP in Human, Mouse samples.

What is the molecular weight of HIF-1 alpha?
Anti-HIF-1 alpha [EPR16897-145] (ab308433) specifically detects a band for HIF-1 alpha (UniProt: Q16665) at a molecular weight of 92kDa.

Specificity confirmed

The specificity of Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433) has been confirmed by Immunocytochemistry/ Immunofluorescence testing in HIF1A Knockout HCT 116 cell line, ab255394.

Other related products
We have a range of other formats of antibody clone [EPR16897-145] also available for your convenience: ab308433, Carrier free - ab308434

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.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

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

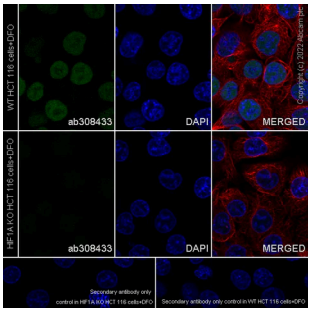
We do not recommend this combination. It is not covered by our product promise.

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.

16 product images

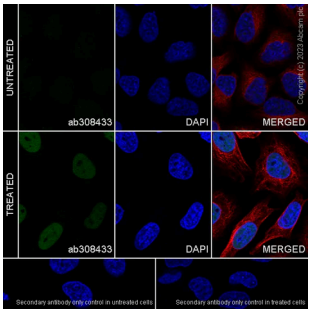


Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433)

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

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HIF1A KO HCT 116 (HIF1A knockout human colorectal carcinoma epithelial cell), ab255394 cells labelling HIF-1 alpha with ab308433 at 1/100 (10.25 ug/ml) dilution, followed by ab150081 Goat

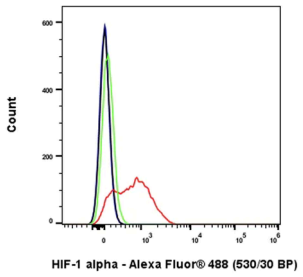
Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2 ug/ml dilution (Green). Confocal images showing nuclear staining in wildtype HCT 116 cells treated with DFO (0.5mM) for 24 hours and showing no staining in HIF1A knockout HCT 116 cells treated with the same condition.Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8). ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Red). The Nuclear counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/ml dilution.



Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433)

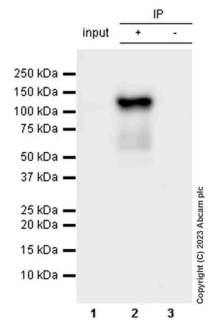
HIF-1 alpha Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HIF-1 alpha antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa (human cervical adenocarcinoma epithelial cell) cells labelling HIF-1 alpha with ab308433 at 1/100 (5.125 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2 ug/ml dilution (Green). Confocal images showing nuclear staining in HeLa cells treated with DFO (0.5 mM) for 24 hours and showing no staining in untreated HeLa cells.Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Red). The Nuclear counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/ml dilution.



Flow Cytometry (Intracellular) - Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (human cervical adenocarcinoma epithelial cell) treated with 0.5mM DFO for 24h (Red) / untreated HeLa (Green) cells labelling HIF-1 alpha with ab308433 at 1/50 dilution (1 ug)/Red and Green (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433)

HIF-1 alpha was immunoprecipitated from 0.35 mg HCT116 (human colorectal carcinoma epithelial cell) treated with 0.5 mM DFO for 24 hour whole cell lysate with ab308433 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab308433 at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/5000 dilution.
Lane 1: HCT116 (human colorectal carcinoma epithelial cell) treated with 0.5 mM DFO for 24 hour whole cell lysate
Lane 2: ab308433 IP in HCT116 (human colorectal carcinoma epithelial cell) treated with 0.5 mM DFO for 24 hour whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab308433 in HCT116 treated with 0.5 mM DFO for 24 hour whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 15 seconds
All lanes:
Immunoprecipitation - Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433) at 1/1000 dilution

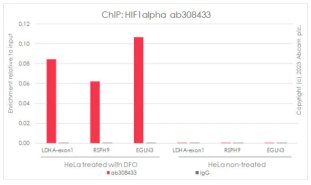
All lanes:
HCT116 (human colorectal carcinoma epithelial cell) treated with 0.5 mM DFO for 24 hour whole cell lysate at 10 µg

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

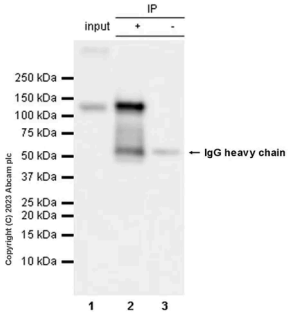
Observed band size: 110 kDa

Exposure time: 15s



ChIP - Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433)

Chromatin was prepared from HeLa (human cervical adenocarcinoma epithelial cell) treated with DFO(0.5mM 24h) and HeLa non-treated according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min. The ChIP was performed with 25 µg of chromatin, 5 µg of ab308433 (red), or 5 µg of rabbit normal IgG ab172730 (gray) and 25 µl of Protein A/G Dynabeads. The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach). Primers are from paper PMID:28193910, 21447827 *Abcam Dual-X-ChIP protocol link



Immunoprecipitation - Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433)

HIF-1 alpha was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell) treated with 0.5 mM DFO for 24 hour whole cell lysate with ab308433 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab308433 at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/5000 dilution.
Lane 1: HeLa (human cervix adenocarcinoma epithelial cell) treated with 0.5 mM DFO for 24 hour whole cell lysate
Lane 2: ab308433 IP in HeLa (human cervix adenocarcinoma epithelial cell) treated with 0.5 mM DFO for 24 hour whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab308433 in HeLa treated with 0.5 mM DFO for 24 hour whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds

All lanes:
Immunoprecipitation - Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433) at 1/1000 dilution

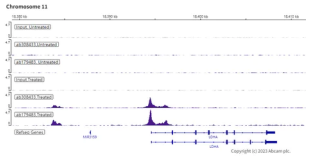
All lanes:
HeLa (human cervix adenocarcinoma epithelial cell) treated with 0.5 mM DFO for 24 hour whole cell lysate at 10 µg

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

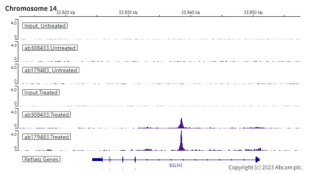
Observed band size: 110 kDa

Exposure time: 10s



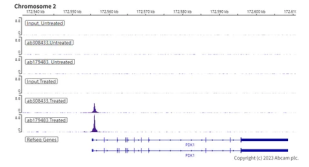
ChIP-sequencing - Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433)

Chromatin was prepared from HeLa cells treated with CoCl2 (350µM 20h+4h) add MG-132(10µM 4h). Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 cells and 8 µg of ab308433 [EPR16897-145] or ab179483 [EPR16897]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads. The Input control is also shown.



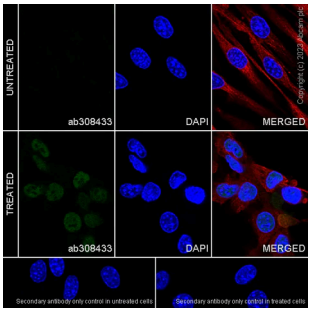
ChIP-sequencing - Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433)

Chromatin was prepared from HeLa cells treated with CoCl2 (350µM 20h+4h) add MG-132(10µM 4h). Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 cells and 8 µg of ab308433 [EPR16897-145] or ab179483 [EPR16897]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads. The Input control is also shown.



ChIP-sequencing - Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433)

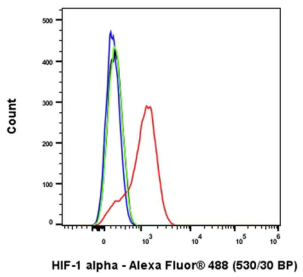
Chromatin was prepared from HeLa cells treated with CoCl2 (350µM 20h+4h) add MG-132(10µM 4h). Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 cells and 8 µg of ab308433 [EPR16897-145] or ab179483 [EPR16897]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads. The Input control is also shown.



Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433)

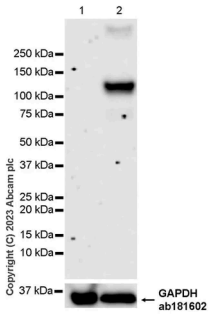
HIF-1 alpha Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HIF-1 alpha antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized bEnd.3 (mouse brain endothelial cell) cells labelling HIF-1 alpha with ab308433 at 1/100 (5.125 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2 ug/ml dilution (Green). Confocal images showing nuclear staining in bEnd.3 cells treated with DFO (2 mM) for 24 hours and showing no staining in untreated bEnd.3 cells.Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Red). The Nuclear counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/ml dilution.



Flow Cytometry (Intracellular) - Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized bEND.3 (mouse brain endothelial cell) treated with 2mM DFO for 24h (Red) / untreated bEND.3 (Green) cells labelling HIF-1 alpha with ab308433 at 1/50 dilution (1 ug)/Red and Green (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Western blot - Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433)

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
The expression of HIF-1 alpha is upregulated in response to DFO treatment (PMID: 25075254).

In Western blot, anti-GAPDH antibody (ab181602) loading control staining at 1/200000 dilution.

This blot was developed using a high sensitivity ECL substrate. The high-sensitivity ECL substrate used allows for the detection of proteins in the mid-femtogram range.

Lysates were freshly made and used for Western blotting immediately to minimize protein degradation.

All lanes:
Western blot - Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433) at 1/1000 dilution

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

Lane 2:
HeLa treated with 0.5 mM DFO for 24 hour whole cell lysate, at 20 µg

Secondary

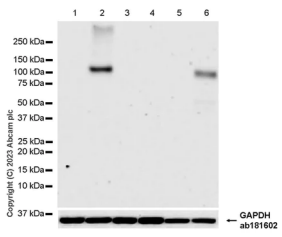
All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 92 kDa

Observed band size: 110 kDa

Exposure time: 180s



Western blot - Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433)

Blocking and diluting buffer and concentration: 5% NFDM/TBST.#Lysates/proteins at 20 µg per lane. Performed under reducing conditions. In Western blot, ab308433 was shown to bind specifically to HIF-1 alpha. A band was observed at 110 kDa in wild-type HCT116 cell lysates with whereas no signal observed at this size in HIF-1 alpha knockout cell line ab255394 (knockout cell lysate). The expression of HIF-1 alpha is upregulated in response to DFO treatment (PMID: 25075254). In Western blot, anti-GAPDH antibody (ab181602) loading control staining at 1/200000 dilution. Lysates were freshly made and used for Western blotting immediately to minimize protein degradation. Exposure time: 180 seconds.
All lanes:
Western blot - Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433) at 1/1000 dilution

Lane 1:
Untreated HCT116 (human colorectal carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
HCT116 treated with 0.5 mM DFO for 24 hour whole cell lysate at 20 µg

Lane 3:
Untreated HIF-1 alpha knockout HCT116 whole cell lysate at 20 µg

Lane 4:
HIF-1 alpha knockout HCT116 treated with 0.5 mM DFO for 24 hour whole cell lysate at 20 µg

Lane 5:
Untreated bEnd.3 (mouse brain endothelioma) whole cell lysate at 20 µg

Lane 6:
bEnd.3 treated with 0.5 mM DFO for 24 hour whole cell lysate at 20 µg

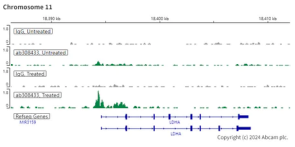
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 92 kDa

Observed band size: 110 kDa

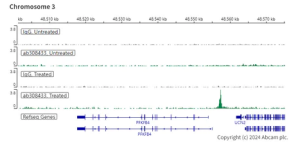
Exposure time: 180s



ChIC/CUT&RUN sequencing - Anti-HIF-1 alpha antibody [EPR16897-145] (ab308433)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL, 2.5 x 10^5 HeLa (Human epithelial cell line from cervix adenocarcinoma) cells treated with CoCl2 (350µM 20h+4h) add MG-132(10µM 4h) and 5 µg of ab308433 [EPR16897-145]. 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.

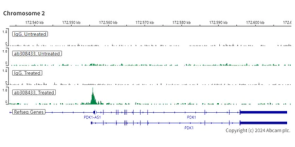
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