


Product datasheet

Anti-HIF-2-alpha antibody ab109616

KO VALIDATED

★★★★☆ 3 Abreviews 23 References 3 Images

Overview

Product name	Anti-HIF-2-alpha antibody
Description	Rabbit polyclonal to HIF-2-alpha
Host species	Rabbit
Specificity	From Jan 2024, QC testing of replenishment batches of this polyclonal changed. All tested and expected application and reactive species combinations are still covered by our Abcam product promise. However, we no longer test all applications. For more information on a specific batch, please contact our Scientific Support who will be happy to help.
Tested applications	Suitable for: WB, IHC-P, ICC/IF
Species reactivity	Reacts with: Mouse, Rat, Human Predicted to work with: Chimpanzee, Macaque monkey, Chinese hamster, Orangutan 
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Wild-type A549 + DFO (1mM, 24 hours) cell lysate. IHC: Human colon tissue. ICC/IF: HeLa cells (untreated and treated with Deferoxamine).
General notes	<p>The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.</p> <p>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab109616 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
WB	★★★★★ (2)	Use a concentration of 1 µg/ml. Detects a band of approximately 100 kDa (predicted molecular weight: 96 kDa).
IHC-P		Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use a concentration of 10 µg/ml.

Target

Function	Transcription factor involved in the induction of oxygen regulated genes. Binds to core DNA sequence 5'-[AG]CGTG-3' within the hypoxia response element (HRE) of target gene promoters. Regulates the vascular endothelial growth factor (VEGF) expression and seems to be implicated in the development of blood vessels and the tubular system of lung. May also play a role in the formation of the endothelium that gives rise to the blood brain barrier. Potent activator of the Tie-2 tyrosine kinase expression. Activation seems to require recruitment of transcriptional coactivators such as CREBPB and probably EP300. Interaction with redox regulatory protein APEX seems to activate CTAD.
Tissue specificity	Expressed in most tissues, with highest levels in placenta, lung and heart. Selectively expressed in endothelial cells.
Involvement in disease	Defects in EPAS1 are the cause of erythrocytosis familial type 4 (ECYT4) [MIM:611783]. ECYT4 is an autosomal dominant disorder characterized by increased serum red blood cell mass, elevated hemoglobin concentration and hematocrit, and normal platelet and leukocyte counts.
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.
Post-translational modifications	In normoxia, is probably hydroxylated on Pro-405 and Pro-531 by EGLN1/PHD1, EGLN2/PHD2 and/or EGLN3/PHD3. The hydroxylated prolines promote interaction with VHL, initiating rapid ubiquitination and subsequent proteasomal degradation. Under hypoxia, proline hydroxylation is impaired and ubiquitination is attenuated, resulting in stabilization. In normoxia, is hydroxylated on Asn-847 by HIF1AN thus probably abrogating interaction with

CREBBP and EP300 and preventing transcriptional activation.

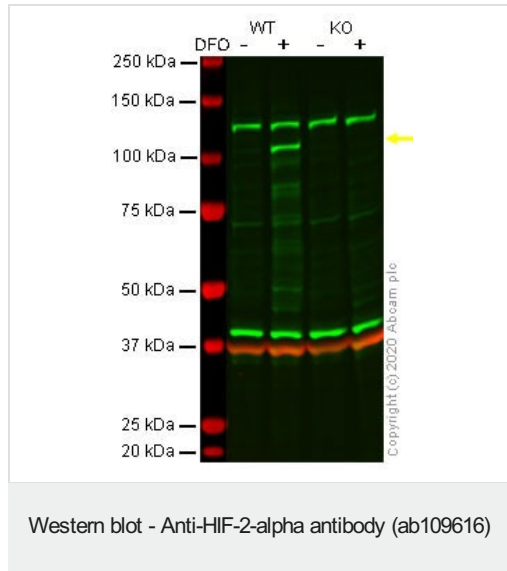
Phosphorylated on multiple sites in the CTAD.

The iron and 2-oxoglutarate dependent 3-hydroxylation of asparagine is (S) stereospecific within HIF CTAD domains.

Cellular localization

Nucleus.

Images



All lanes : Anti-HIF-2-alpha antibody (ab109616) at 1 µg/ml

Lane 1 : Wild-type A549 untreated cell lysate

Lane 2 : Wild-type A549 + DFO (1mM, 24 hours) cell lysate

Lane 3 : EPAS knockout A549 untreated cell lysate

Lane 4 : EPAS knockout A549 + DFO (1mM, 24 hours) cell lysate

Lysates/proteins at 40 µg per lane.

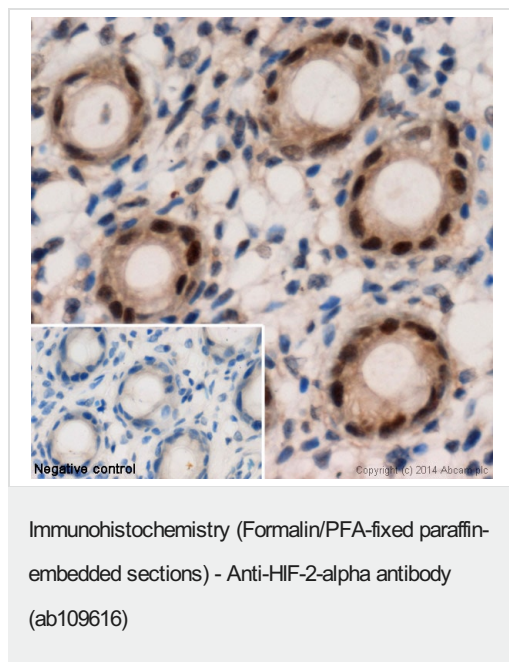
Performed under reducing conditions.

Predicted band size: 96 kDa

Observed band size: 100 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab109616 observed at 100 kDa. Red - loading control [ab8245](#) (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

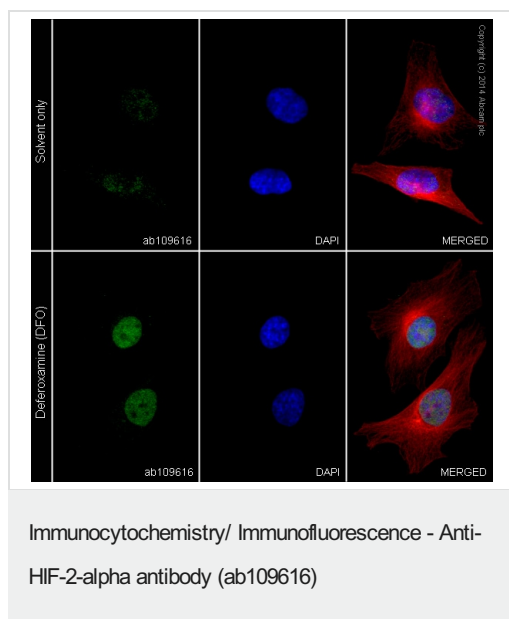
ab109616 was shown to react with HIF-2-alpha in A549 wild-type cells in western blot with loss of signal observed in EPAS1 knockout sample. A549 wild-type and EPAS1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab109616 and [ab8245](#) (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at 1 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ([ab216773](#)) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ([ab216776](#)) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



IHC image of HIF-2-alpha staining in a section of formalin-fixed paraffin-embedded normal human colon*, performed on a Leica BOND. The section was pre-treated using heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 for 20mins. The section was then incubated with ab109616 at 1µg/ml, for 15 mins at room temperature. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset negative control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



ab109616 staining Hif-2-alpha in HeLa cells. The cells were incubated with 1mM Deferoxamine for 24 hours (Treated) or solvent-only for control purposes (Solvent only). Cells were fixed with 4% formaldehyde (10min) 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 ab109616 at 10µg/ml and **ab195889** at 1µg/ml overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

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