abcam

Product datasheet

Anti-HIF-2-alpha antibody [EPR19656] - BSA and Azide free ab222396

Recombinant RabMAb

3 Images

Overview

Product name	Anti-HIF-2-alpha antibody [EPR19656] - BSA and Azide free
Description	Rabbit monoclonal [EPR19656] to HIF-2-alpha - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IP, WB, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Whole cell lysate of HeLa cells treated with 1mM deferoxamine (DFO) mesylate salt for 24h ; Whole cell lysate of HeLa cells treated with 100µM CoCl2 for 24h. ICC/IF: HeLa cells treated with DFO mesylate salt (1mM, 24h). IP: HeLa cell lysate.
General notes	ab222396 is the carrier-free version of **ab207607**. 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. 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.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR19656
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab222396 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
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 110 kDa (predicted molecular weight: 96 kDa).
ICC/IF		Use at an assay dependent concentration.

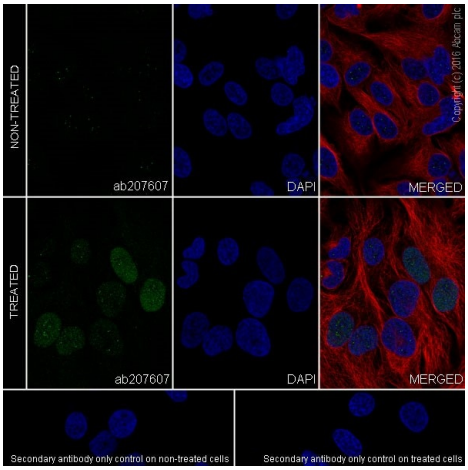
Target

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



Immunocytochemistry/ Immunofluorescence - Anti-HIF-2-alpha antibody [EPR19656] - BSA and Azide free (ab222396)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells, untreated or treated with deferoxamine mesylate salt (1mM, 24h), labeling HIF-2-alpha with **ab207607** at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

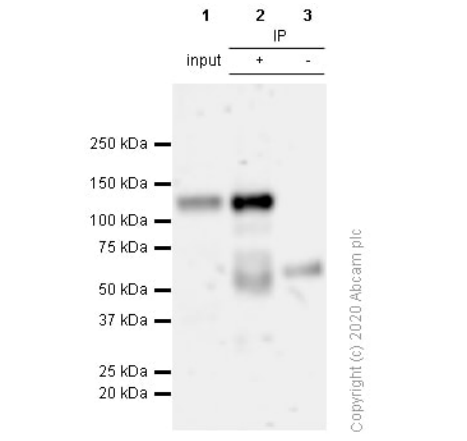
Confocal image showing nuclear staining on HeLa cells treated with deferoxamine mesylate salt (1mM, 24h).

The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207607**).



Immunoprecipitation - Anti-HIF-2-alpha antibody [EPR19656] - BSA and Azide free (ab222396)

HIF-2-alpha was immunoprecipitated from 0.35 mg HeLa (Human cervix adenocarcinoma epithelial cell) cell treated with 1mM DFO for 24h, cell lysate 10 µg with **ab207607** at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using **ab207607** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(**ab131366**) was used at 1/5000 dilution.

Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) cell lysate (10 µg) treated with 1mM DFO for 24h

Lane 2: **ab207607** IP in DFO treated HeLa cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab207607** in HeLa cell lysate

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

Exposure time: 75 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207607**).

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