abcam

Product datasheet

Alexa Fluor® 647 Anti-HIF-1 alpha antibody [EPR16897] ab208420



2 References 2 Images

Overview

Product name Alexa Fluor® 647 Anti-HIF-1 alpha antibody [EPR16897]

Description Alexa Fluor® 647 Rabbit monoclonal [EPR16897] to HIF-1 alpha

Host species Rabbit

Conjugation Alexa Fluor® 647. Ex: 652nm, Em: 668nm

Tested applications
Suitable for: ICC/IF
Species reactivity
Reacts with: Human

Predicted to work with: Mouse

Immunogen Recombinant fragment within Human HIF-1 alpha aa 300-550. The exact immunogen sequence

used to generate this antibody is proprietary information. If additional detail on the immunogen is needed to determine the suitability of the antibody for your needs, please **contact** our Scientific

Support team to discuss your requirements.

Database link: Q16665

Run BLAST with
Run BLAST with

Positive control ICC/IF: DFO-treated HeLa cells

General notesThis 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**.

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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.

Avoid freeze / thaw cycle. Store In the Dark.

Storage buffer pH: 7.40

Preservative: 0.02% Sodium azide

Constituents: PBS, 1% BSA, 30% Glycerol (glycerin, glycerine)

Purity Protein A purified

ClonalityMonoclonalClone numberEPR16897

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab208420 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 | | 1/50. This product gave a positive signal in DFO-treated HeLa cells fixed with 4% formaldehyde (10 min) |

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.

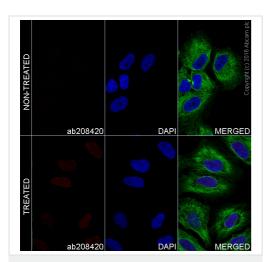
Sumoylated; by SUMO1 under hypoxia. Sumoylation is enhanced through interaction with RWDD3. Desumoylation by SENP1 leads to increased HIF1A stability and transriptional activity. Ubiquitinated; in normoxia, following hydroxylation and interaction with VHL. Lys-532 appears to be the principal site of 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.

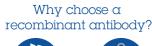
Images



Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 647 Anti-HIF-1 alpha antibody [EPR16897] (ab208420) ab208420 staining HIF-1-alpha in HeLa cells +/- DFO (1nM, 24 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 ab208420 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).





Research with confidence Consistent and reproducible results









Confirmed Animal-free specificity production

Alexa Fluor® 647 Anti-HIF-1 alpha antibody [EPR16897] (ab208420)

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