abcam

Product datasheet

Anti-HIF-1 alpha antibody [ESEE122] ab8366

★★★★★ 2 Abreviews 52 References 2 图像

概述

产品名称 Anti-HIF-1 alpha抗体[ESEE122]

描述 小鼠单克隆抗体[ESEE122] to HIF-1 alpha

宿主 Mouse

经测试应用 **适用于:** ICC/IF, IHC-P

不适用于: WB

种属反应性 与反应: Human

免疫原 Recombinant fragment corresponding to Human HIF-1 alpha aa 300-550.

阳性对照 IHC-P: Human colon tissue. ICC: HeLa BrdU cells.

常规说明 Under normoxic conditions HIF-1 alpha has a short half-life. It is largely undetectable in cells or

tissues grown under normoxic conditions. It is stabilized only at O_2 concentrations below 5% and upon stabilization under hypoxic conditions HIF-1 translocates to the nucleus. Therefore we recommend western blots using nuclear extracts and running Hypoxia treated samples as positive

control (ab180880). Hypoxia can be induced with treatment using certain agents e.g. CoCl₂ or

DFO, etc. so proper sample preparation is critical.

This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or

conjugation for your experiments, please contact $\underline{ orders@abcam.com}.$

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.

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

性能

形式 Liquid

存放说明 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

存储溶液 pH: 7.40

Preservative: 0.02% Sodium azide

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Constituents: PBS, 6.97% L-Arginine

纯**度** Protein G purified

克隆 单克隆

克隆编号 ESEE122

骨髓瘤 NS1 **同种型** IqG1

轻链类型 unknown

应用

The Abpromise guarantee Abpromise™承诺保证使用ab8366于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF		Use a concentration of 8 - 12 µg/ml.
IHC-P	★★★★ (2)	1/1000 - 1/8000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

应用说明 ls unsuitable for WB.

靶标

功能 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.

组织特异性 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.

序列相似性 Contains 1 basic helix-loop-helix (bHLH) domain.

Contains 1 PAC (PAS-associated C-terminal) domain.

Contains 2 PAS (PER-ARNT-SIM) domains.

结**构域** Contains two independent C-terminal transactivation domains, NTAD and CTAD, which function

synergistically. Their transcriptional activity is repressed by an intervening inhibitory domain (ID).

翻译后修饰 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.

Cytoplasm. Nucleus. Cytoplasmic in normoxia, nuclear translocation in response to hypoxia. Colocalizes with SUMO1 in the nucleus, under hypoxia.

细胞定位

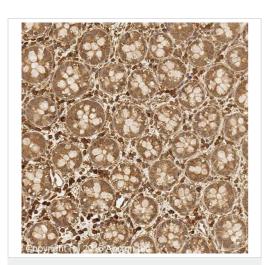
图片

MERGED ab8366

Immunocytochemistry/ Immunofluorescence - Anti-HIF-1 alpha antibody [ESEE122] (ab8366)

ab8366 staining HIF-1 alpha in Hela cells. Untreated and BrdU treated (10µM for 24 hours) cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-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 ab8366 at 10µg/ml and ab6046, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with ab150117, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and ab150080, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HIF-1 alpha antibody
[ESEE122] (ab8366)

IHC image of HIF-1-alpha staining in human normal colon formalin fixed paraffin embedded tissue section*, performed on a Leica Bond™ system using the standard protocol F. The section was pretreated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab8366, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

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

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