Product datasheet

Anti-HIF-1 alpha antibody [ESEE122] ab8366

Overview

<table>
<thead>
<tr>
<th>Product name</th>
<th>Anti-HIF-1 alpha antibody [ESEE122]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Mouse monoclonal [ESEE122] to HIF-1 alpha</td>
</tr>
<tr>
<td>Host species</td>
<td>Mouse</td>
</tr>
<tr>
<td>Specificity</td>
<td>This antibody is specific for HIF-1-alpha.</td>
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<tr>
<td>Tested applications</td>
<td>Suitable for: Flow Cyt, ICC/IF, ELISA, IHC-Fr, IHC-P</td>
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<tr>
<td></td>
<td>Unsuitable for: WB</td>
</tr>
<tr>
<td>Species reactivity</td>
<td>Reacts with: Mouse, Rat, Cow, Human</td>
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<tr>
<td>Immunogen</td>
<td>Recombinant fragment corresponding to Human HIF-1 alpha aa 300-550.</td>
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<tr>
<td>Positive control</td>
<td>ICC: cultured raw mouse macrophage cells IHC-P: glioblastoma multiformae, hypoxia-induced human placenta, human normal colon</td>
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<tr>
<td>General notes</td>
<td>This antibody clone is manufactured by Abcam.</td>
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</tbody>
</table>

HIF-1 alpha can be a difficult target to work with so we have compiled a summary of all the important information you need to know including useful tips. This can be found in the protocols tab or alternatively click here to download it.

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 O2 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.

If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information here.

Properties

<table>
<thead>
<tr>
<th>Form</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage instructions</td>
<td>Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.</td>
</tr>
<tr>
<td>Storage buffer</td>
<td>pH: 7.4</td>
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<tr>
<td></td>
<td>Preservative: 0.02% Sodium azide</td>
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<tr>
<td></td>
<td>Constituents: PBS, 6.97% L-Arginine</td>
</tr>
</tbody>
</table>
Purity: Immunogen affinity purified
Clonality: Monoclonal
Clone number: ESEE122
Myeloma: NS1
Isotype: IgG1
Light chain type: unknown

Applications

Our Abpromise guarantee covers the use of ab8366 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Cyt</td>
<td>Use 1µg for 10^6 cells.</td>
<td>ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>Use a concentration of 8 - 12 µg/ml.</td>
<td></td>
</tr>
<tr>
<td>ELISA</td>
<td>Use at an assay dependent concentration.</td>
<td></td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>1/1000 - 1/8000.</td>
<td></td>
</tr>
<tr>
<td>IHC-P</td>
<td>1/1000 - 1/8000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
<td></td>
</tr>
</tbody>
</table>

Application notes: Is unsuitable for WB.

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


Images

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

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of hypoxia-induced Human placenta labeling HIF-1-alpha with ab8366.
Detection of HIF-1-alpha (red dye 568) in a cultured raw mouse macrophage cell line, using ab8366.

Photos courtesy of Susan Alexander and Hattie Gresham, PhD.

Overlay histogram showing HeLa cells stained with ab8366 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab8366, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.
ab8366 stained Hela cells. The cells were 4% formaldehyde fixed for 10 minutes, permeabilized in 0.1% PBS-Triton X-100 for 5 min and then blocked in 1% BSA / 10% normal Goat serum / 0.3M glycine in 0.1% PBS-Tween for 1 hour at room temperature to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab8366 at 10μg/ml) overnight at +4°C. The secondary antibody (pseudo-colored green) was ab150117 Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed used at a 1/1000 dilution for 1hour at room temperature. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1hour at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43μM for 1hour at room temperature.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded 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 pre-treated 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

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

Detection of HIF-1-alpha (red dye) in a cell cytospin from a lavage of a murine skin pouch infected with Staph Aureus, using ab8366. 100X magnification. Blue dye is DAPI nuclear staining.

Photos courtesy of Susan Alexander and Hattie Gresham, PhD.
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Detection of HIF-1-alpha (red dye 568) in a cultured raw mouse macrophage cell line, using ab8366. 100X magnification.

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