abcam

Product datasheet

Anti-HIF-2-alpha antibody [BL-95-1A2] ab243861

Recombinant

5 References 7 Images

Overview

Product name	Anti-HIF-2-alpha antibody [BL-95-1A2]
Description	Rabbit monoclonal [BL-95-1A2] to HIF-2-alpha
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, IP, ChIP-sequencing, ICC, IHC-P, WB
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human HIF-2-alpha aa 400-450. The exact sequence is proprietary. NP_001421.2 and Gene ID 2034. Database link: **Q99814**
General notes	This product is sold under License from Bethyl Laboratories, Inc.

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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.8 Preservative: 0.09% Sodium azide Constituents: 98% Borate buffered saline, 0.1% BSA
Purification notes	Recombinant antibody was purified from cell culture supernatant.
Clonality	Monoclonal
Clone number	BL-95-1A2
Isotype	IgG

Applications

The Abpromise guarantee	Our **Abpromise guarantee** covers the use of ab243861 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		1/100 - 1/500. Permeabilization with Triton-X 100 is recommended for formaldehyde-fixed cells.
IP		Use at an assay dependent concentration. Use 5-20µl/mg lysate.
ChIP-sequencing		Use a concentration of 10-40 - 30 µl/chromatin.
ICC		1/200 - 1/1000. Permeabilization with Triton-X 100 is recommended for formaldehyde-fixed cells.
IHC-P		1/200 - 1/1000. Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol. Antigen retrieval with citrate buffer pH 6.0 for 20 minutes using a pressure cooker and overnight incubations are recommended.
WB		1/1000.

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

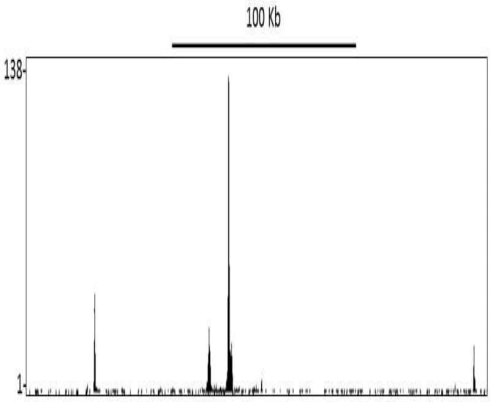
Contains 1 basic helix-loop-helix (bHLH) domain.
Contains 1 PAC (PAS-associated C-terminal) domain.
Contains 2 PAS (PER-ARNT-SIM) domains.

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.

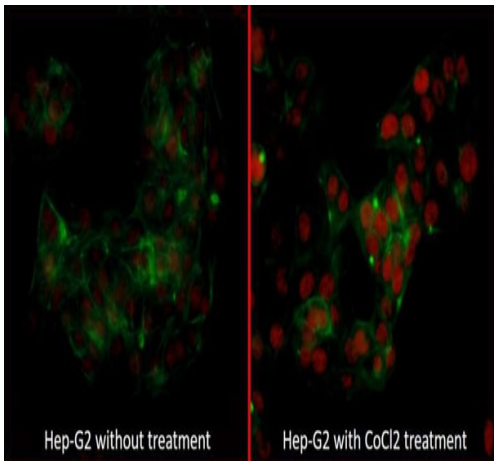
Cellular localization Nucleus.

Images



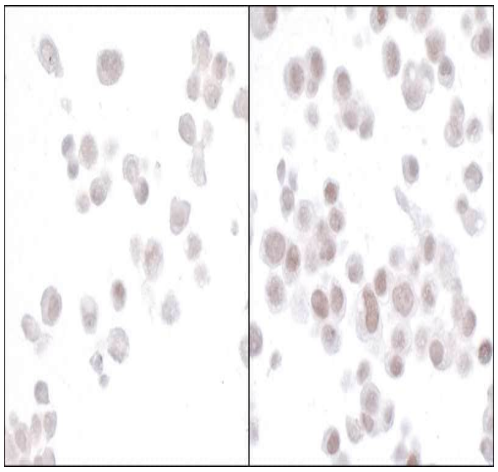
ChIP-sequencing - Anti-HIF-2-alpha antibody [BL-95-1A2] (ab243861)

Chromatin from subcutaneous human tumor 786-O cells was immunoprecipitated with anti-HIF-2-alpha antibody ab243861 and analyzed by
DNA sequencing. The figure illustrates the peak distribution of HIF-2-alpha binding within a 250 Kb region of chromosome 11 as detected
using anti-HIF-2-alpha antibody ab243861. ChIP-seq validation performed by Active Motif, Carlsbad, CA.



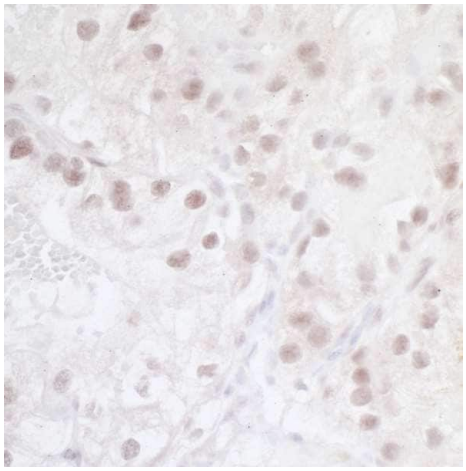
Immunocytochemistry/ Immunofluorescence - Anti-HIF-2-alpha antibody [BL-95-1A2] (ab243861)

Formaldehyde-fixed HepG2 (human liver hepatocellular carcinoma cell line) cells untreated (Left) and treated with CoCl2 (right), labeling HIF-2 alpha (Red) using ab243861 at 1/100 dilution. DyLight® 594-conjugated goat anti-rabbit IgG was used as the secondary antibody at 1/100 dilution. Phalloidin Alexa Fluor® 488conjugated (green) was used as the counterstain.



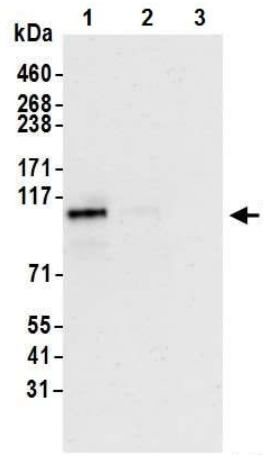
Immunocytochemistry - Anti-HIF-2-alpha antibody [BL-95-1A2] (ab243861)

Formalin-fixed, paraffin-embedded HepG2 (human liver hepatocellular carcinoma cell line) cells labeling HIF-2 alpha using ab243861 at 1/400 dilution in ICC/IF analysis. A HRP-conjugated goat-anti rabiit IgG was used as the secondary. DAB staining.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF-2-alpha antibody [BL-95-1A2] (ab243861)

Formalin-fixed, paraffin-embedded human renal cell carcinoma tissue stained for HIF-2 alpha using ab243861 at 1/400 dilution in immunohistochemical analysis. A HRP-conjugated goat anti-rabbit IgG was used as the secondary. DAB staining.



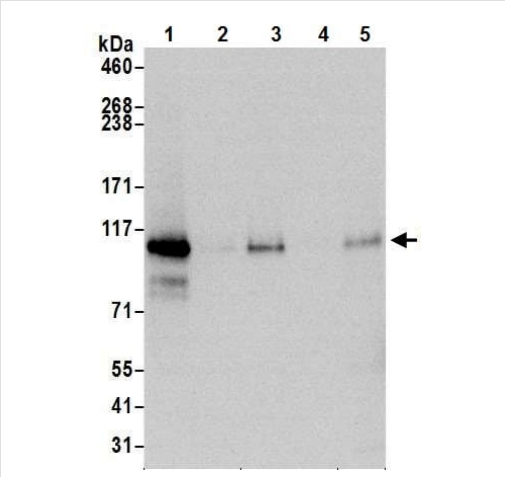
Immunoprecipitation - Anti-HIF-2-alpha antibody [BL-95-1A2] (ab243861)

HIF-2 alpha was immunoprecipitated from 1 mg HepG2 whole cell lysate with ab243861 at 20 µL per reaction. Western blot was performed on the immunoprecipitate using ab243861 at 1/1000 dilution.

Lane 1: ab243861 IP in HepG2 cell lysate treated with 200 µM CoCl2.

Lane 2: ab243861 IP in HepG2 whole cell lysate Mock treated.

Lane 3: Contol IgG in HepG2 cell lysate treated with 200 µM CoCl2.



Western blot - Anti-HIF-2-alpha antibody [BL-95-1A2] (ab243861)

All lanes : Anti-HIF-2-alpha antibody [BL-95-1A2] (ab243861) at 1/1000 dilution

Lane 1 : HepG2 whole cell lysate treated with 200uM CoCl2

Lane 2 : HepG2 whole cell lysate mock treated

Lane 3 : HEK293T whole cell lysate treated with 200uM CoCl2

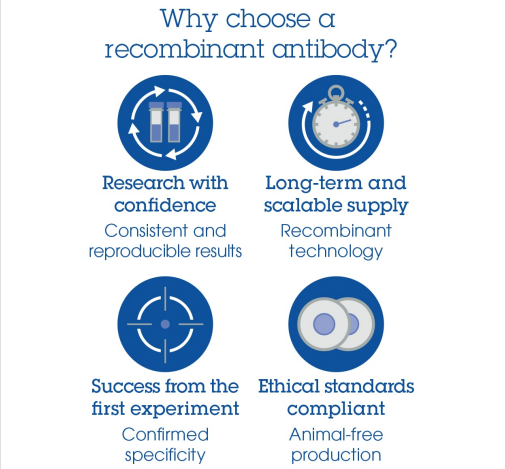
Lane 4 : HEK293T whole cell lysate mock treated

Lane 5 : 786-O whole cell lysate mock treated

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : HRP-conjugated goat anti-rabbit IgG



Anti-HIF-2-alpha antibody [BL-95-1A2] (ab243861)

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