abcam

Product datasheet

Human EPAS1 (HIF-2-alpha) knockout A549 cell line ab259774

3 Images

Overview

Product name	Human EPAS1 (HIF-2-alpha) knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 1 bp insertion; Frameshift = 99%
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS), Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	1
General notes	**Recommended control:** Human wild-type A549 cell line (**ab259777**). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM:Hams F12 + 5% FBS **Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability. 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution. 3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $2x10^3$-$1x10^4$ cells/cm^2. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $6x10^4$ cells/cm^2 is recommended.

A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.
Cells should be passaged when they have achieved 80-90% confluence.
Do not exceed $7x10^4$ cells/cm^2.
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We will provide viable cells that proliferate on revival.

Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Lung
Cell type	epithelial
Disease	Carcinoma
Gender	Male
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

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.

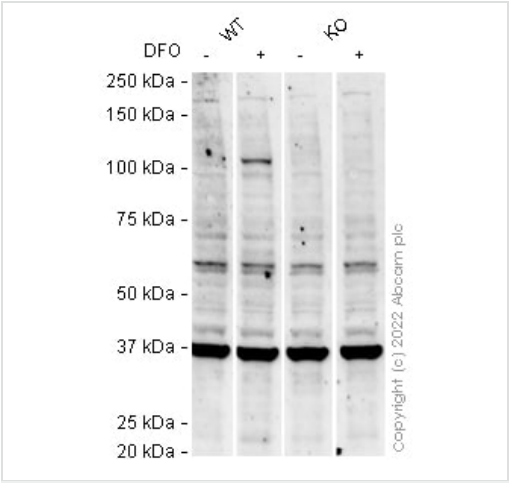
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab259774 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
WB		Use at an assay dependent concentration.

Images



Western blot - Human EPAS1 (HIF-2-alpha) knockout A549 cell line (ab259774)

All lanes : Anti-HIF-2-alpha antibody [OTI2G5] (**ab157249**) at 1/500 dilution

Lane 1 : Wild-type A549 Untreated (DFO Control) cell lysate
Lane 2 : Wild-type A549 Treated DFO (1 mM, 24 h) cell lysate
Lane 3 : EPAS1 knockout A549 Untreated (DFO Control) cell lysate
Lane 4 : EPAS1 knockout A549 Treated DFO (1 mM, 24 h) cell lysate

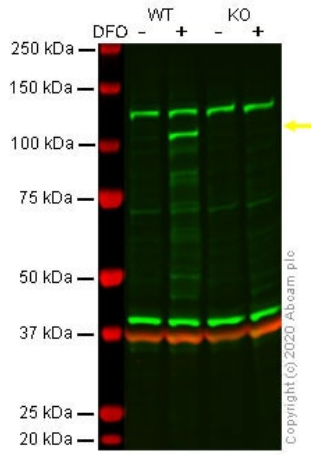
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 100 kDa

False colour image of Western blot: Anti-HIF-2-alpha antibody [OTI2G5] staining at 1/500 dilution, shown in black; Rabbit Anti-GAPDH antibody [EPR16891] (**ab181602**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab157249** was shown to bind specifically to HIF-2-alpha. A band was observed at 100 kDa in treated wild-type A549 cell lysates with no signal observed at this size in EPAS1 knockout cell line ab259774 (knockout cell lysate **ab259779**). To generate this image, wild-type and EPAS1 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a

nitrocellulose membrane. Membranes were blocked in 5 % BSA in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times before development with Optiblot (ECL reagent **ab133456**) and imaged with 20 seconds exposure time. Secondary antibodies used were HRP conjugated Goat anti-Mouse (H+L) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) at 1/20000 dilution.



Western blot - Human EPAS1 (HIF-2-alpha) knockout A549 cell line (ab259774)

All lanes : Anti-HIF-2-alpha antibody (**ab109616**) at 1 µg/ml

Lane 1 : Wild-type A549 untreated cell lysate
Lane 2 : Wild-type A549 + DFO (1mM, 24 hours) cell lysate
Lane 3 : EPAS knockout A549 untreated cell lysate
Lane 4 : EPAS knockout A549 + DFO (1mM, 24 hours) cell lysate

Lysates/proteins at 40 µg per lane.

Performed under reducing conditions.

Observed band size: 100 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab109616** observed at 100 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab109616 was shown to react with HIF-2-alpha in A549 wild-type cells in western blot with loss of signal observed in EPAS1 knockout cell line ab259774 (knockout cell lysate **ab259779**). A549 wild-type and EPAS1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab109616** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at 1 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Next Generation Sequencing - Human EPAS1 (HIF-2-alpha) knockout A549 cell line (ab259774)

X = 1 bp insertion

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