abcam

Product datasheet

Human EIF2AK2 (PKR) knockout A549 cell line ab266999

4 Images

Overview

Product name	Human EIF2AK2 (PKR) knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon 5 and 1 bp insertion in exon 5
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	1
General notes	**Recommended control:** Human wild-type A549 cell line (ab255450). 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:** F-12K + 10% FBS **Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80ºC. Storage at -80ºC may result in loss of viability. 1. Thaw the vial in 37ºC water bath 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 (Click here to view haemocytometer protocol) or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $2x10^4$ cells/cm^2. This should allow for confluency within 48 hours. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37ºC 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 $2x10^4$ cells/cm^2 is recommended for confluency (80-90% confluence) within 48 hours.
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.
Click here to view the Mammalian cell tissue culture protocol

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Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Viability	~90%
Adherent /Suspension	Adherent
Tissue	Lung
Cell type	epithelial
Disease	Carcinoma
Gender	Male
STR Analysis	Amelogenin X,Y D5S818: 11 D13S317: 11 D7S820: 8, 11 D16S539: 11, 12 vWA: 14 TH01: 8,9.3 TPOX: 8,11 CSF1PO: 10, 12
Antibiotic resistance	Puromycin 1.00µg/ml
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% DMSO, 2% Cellulose, methyl ether

Target

Function	Following activation by double-stranded RNA in the presence of ATP, the kinase becomes autophosphorylated and can catalyze the phosphorylation of the translation initiation factor EIF2S1, which leads to an inhibition of the initiation of protein synthesis. Double-stranded RNA is generated during the course of a viral infection.
Sequence similarities	Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. GCN2 subfamily. Contains 2 DRBM (double-stranded RNA-binding) domains. Contains 1 protein kinase domain.
Post-translational modifications	Autophosphorylated on several Ser and Thr residues. Autophosphorylation of Thr-451 is dependent on Thr-446 and is stimulated by dsRNA binding and dimerization. Autophosphorylation apparently leads to the activation of the kinase.

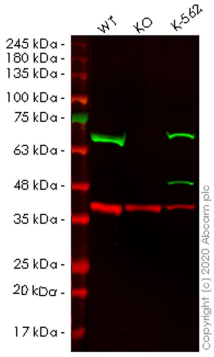
Applications

Our Abpromise guarantee covers the use of **ab266999** 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. Predicted molecular weight: 62 kDa.

Images



Western blot - Human EIF2AK2 knockout A549 cell line (ab266999)

All lanes : Anti-PKR antibody [YE350] (ab32052) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : EIF2AK2 knockout A549 cell lysate
Lane 3 : K-562 cell lysate

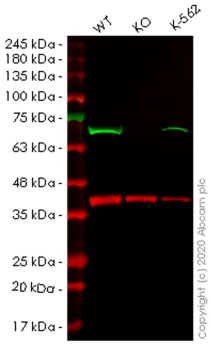
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 62 kDa
Observed band size: 70 kDa
why is the actual band size different from the predicted?

Lanes 1-3: Merged signal (red and green). Green - ab32052 observed at 70 kDa. Red - loading control ab8245 observed at 36 kDa.

ab32052 Anti-PKR antibody [YE350] was shown to specifically react with PKR in wild-type A549 cells. Loss of signal was observed when knockout cell line ab266999 (knockout cell lysate ab256900) was used. Wild-type and PKR knockout samples were subjected to SDS-PAGE. ab32052 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at room temperature for 2.5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human EIF2AK2 knockout A549 cell line (ab266999)

All lanes : Anti-PKR antibody [EPR19374] (ab184257) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : EIF2AK2 knockout A549 cell lysate
Lane 3 : K-562 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 62 kDa
Observed band size: 70 kDa why is the actual band size different from the predicted?

Lanes 1-3: Merged signal (red and green). Green - ab184257 observed at 70 kDa. Red - loading control ab8245 observed at 36 kDa.

ab184257 Anti-PKR antibody [EPR19374] was shown to specifically react with PKR in wild-type A549 cells. Loss of signal was observed when knockout cell line ab266999 (knockout cell lysate ab256900) was used. Wild-type and PKR knockout samples were subjected to SDS-PAGE. ab184257 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at room temperature for 2. 5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Sanger Sequencing - Human EIF2AK2 knockout A549 cell line (ab266999)

Allele-1: 1 bp deletion in exon5

Sanger Sequencing - Human EIF2AK2 knockout A549 cell line (ab266999)

Allele-2: 1 bp insertion in exon 5.

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