abcam

Product datasheet

Human CHEK1 heterozygous knockout A549 cell line ab276102

画像数 4

製品の概要

製品名	Human CHEK1 heterozygous knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Heterozygous: 46 bp deletion in exon 3 and wild-type.
Passage number	<20
Knockout validation	Sanger Sequencing
Biosafety level	1
特記事項	**Recommended control:** Human wild-type A549 cell line (**ab275463**). 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 -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.
This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.

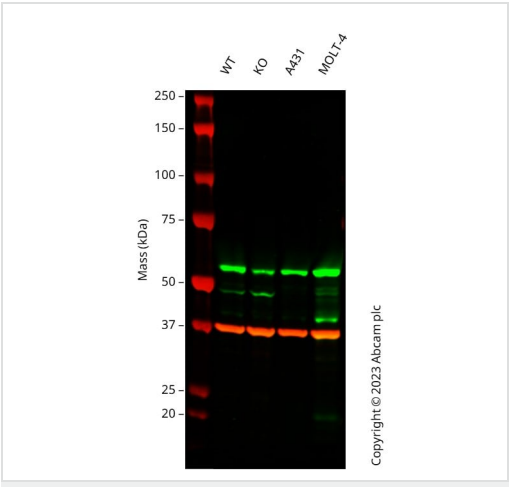
We will provide viable cells that proliferate on revival.

製品の特性

Number of cells	1 x 10^6 cells/vial, 1 mL
Viability	~50%
Adherent /Suspension	Adherent
Tissue	Lung
Cell type	epithelial
Disease	Carcinoma
Gender	Male
Mycoplasma free	Yes
保存方法	Shipped on Dry Ice. Store in liquid nitrogen.
バッファー	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

ターゲット情報

画像



Western blot - Human CHEK1 heterozygous knockout A549 cell line (ab276102)

All lanes : Anti-CHEK1 antibody at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : CHEK1 knockout A549 cell lysate
Lane 3 : A431 cell lysate
Lane 4 : MOLT-4 cell lysate

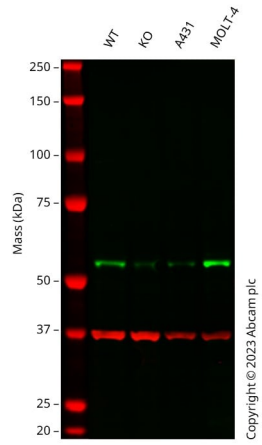
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 57 kDa

Anti-CHEK1 antibody [2G1D5] staining at 1/1000 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (**ab181602**)

loading control staining at 1/20000 dilution, shown in red. In Western blot, this antibody was shown to bind specifically to CHEK1. A band was observed at 57 kDa in wild-type A549 cell lysates with a reduction in signal observed at this size in CHEK1 heterozygous knockout cell line. To generate this image, wild-type and CHEK1 heterozygous 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 % milk 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 then imaged. Secondary antibodies used were Goat anti-Mouse IgG H&L 800CW and Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution.



Western blot - Human CHEK1 heterozygous knockout A549 cell line (ab276102)

All lanes : Anti-Chk1 antibody [EP691Y] (**ab40866**) at 1/10000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : CHEK1 knockout A549 cell lysate
Lane 3 : A431 cell lysate
Lane 4 : MOLT-4 cell lysate

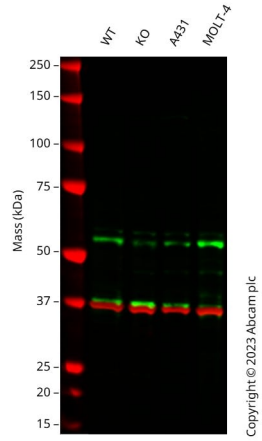
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 57 kDa

Anti-CHEK1 antibody [EP691Y] (**ab40866**) staining at 1/10000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab40866** was shown to bind specifically to CHEK1. A band was observed at 57 kDa in wild-type A549 cell lysates with a reduction in signal observed at this size in CHEK1 heterozygous knockout cell line. To generate this image, wild-type and CHEK1 heterozygous 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 3 % milk 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 then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Human CHEK1 heterozygous knockout A549 cell line (ab276102)

All lanes : Anti-Chk1 antibody [E250] (**ab32531**) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : CHEK1 knockout A549 cell lysate
Lane 3 : A431 cell lysate
Lane 4 : MOLT-4 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 57 kDa

Anti-CHEK1 antibody [E250] (**ab32531**) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab32531** was shown to bind specifically to CHEK1. A band was observed at 57 kDa in wild-type A549 cell lysates with a reduction in signal observed at this size in CHEK1 heterozygous knockout cell line. To generate this image, wild-type and CHEK1 heterozygous 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 3 % milk 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 then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

Sanger Sequencing - Human CHEK1 knockout A549 cell line (ab276102)

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47 bp deletion in exon 3 and wild type.

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