abcam

Product datasheet

Human S100A4 knockout A549 cell line ab261865

6 Images

Overview

Product name	Human S100A4 knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 1 bp insertion; Frameshift = 95%
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS), Western Blot (WB)
Tested applications	**Suitable for:** WB, Next Generation Sequencing
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

Tissue specificity	Ubiquitously expressed.
Sequence similarities	Belongs to the S-100 family. Contains 2 EF-hand domains.

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab261865 in the following tested applications.

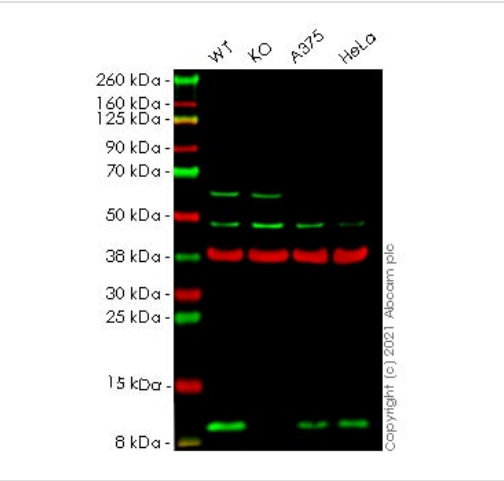
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration.
Next Generation Sequencing		Use at an assay dependent concentration.

Images

Next Generation Sequencing - Human S100A4 knockout A549 cell line (ab261865)

1 bp insertion after His16 of the WT protein



Western blot - Human S100A4 knockout A549 cell line (ab261865)

All lanes : Anti-S100A4 antibody [EPR2761(2)] (**ab124805**) at 1/1000 dilution

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 2 : S100A4 knockout A549 (Human lung carcinoma cell line) whole cell lysate
Lane 3 : A-375 (Human malignant melanoma cell line) whole cell lysate
Lane 4 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

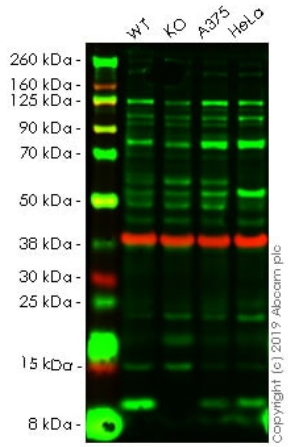
Performed under reducing conditions.

Observed band size: 12 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab124805** observed at 12 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab124805 was shown to react with S100A4 in wild-type A549 cells in Western blot with loss of signal observed in S100A4 knockout cell line ab261865 (knockout cell lysate **ab261674**). Wild-type A549 and S100A4 knockout cell lysates were subjected to SDS-PAGE.

Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab124805** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution 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 h at room temperature before imaging.



Western blot - Human S100A4 knockout A549 cell line (ab261865)

All lanes : Anti-S100A4 antibody (**ab41532**) at 1/250 dilution

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 2 : S100A4 knockout A549 (Human lung carcinoma cell line) whole cell lysate
Lane 3 : A-375 (Human malignant melanoma cell line) whole cell lysate, 20 ug
Lane 4 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

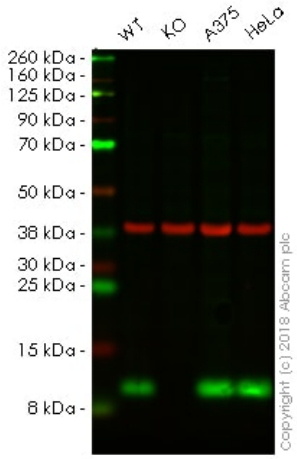
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 12 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab41532** observed at 12 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab41532 was shown to recognize S100A4 in wild-type A549 cells as signal was lost at the expected MW in S100A4 knockout cell line ab261865 (knockout cell lysate **ab261674**). Additional cross-reactive bands were observed in the wild-type and knockout samples. Wild-type and S100A4 knockout samples were subjected to SDS-PAGE. Ab41532 and **ab8245** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/250 dilution and 1/20000 dilution respectively. Blots were developed 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 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human S100A4 knockout A549 cell line (ab261865)

All lanes : Anti-S100A4 antibody [S100A4/1482] (**ab218512**) at 1 µg/ml

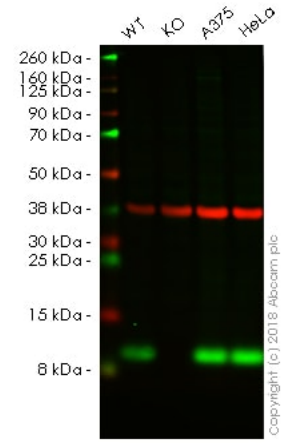
Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 2 : Human S100A4 knockout A549 (Human lung carcinoma cell line) whole cell lysate
Lane 3 : A-375 (Human malignant melanoma cell line) whole cell lysate
Lane 4 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Observed band size: 12 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab218512** observed at 12 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

ab218512 was shown to specifically react with S100A4 in wild-type A549 cells as signal was lost in S100A4 knockout cell line ab261865 (knockout cell lysate **ab261674**). Wild-type and S100A4 knockout samples were subjected to SDS-PAGE. Ab218512 and **ab181602** (Rabbit anti GAPDH loading control) were incubated overnight at 4°C at 1 ug/ml and 1/20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed **ab216772** and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed **ab216777** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human S100A4 knockout A549 cell line (ab261865)

All lanes : Anti-S100A4 antibody [S100A4/1481] (**ab218511**) at 1 µg/ml

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 2 : S100A4 knockout A549 (Human lung carcinoma cell line) whole cell lysate
Lane 3 : A-375 (Human malignant melanoma cell line) whole cell lysate
Lane 4 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

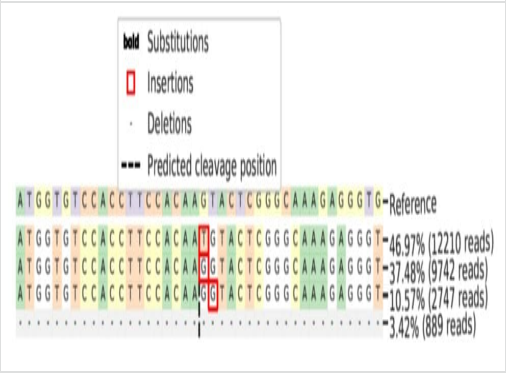
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 12 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab218511** observed at 12 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

ab218511 was shown to specifically react with S100A4 in wild-type A549 cells as signal was lost in S100A4 knockout cell line ab261865 (knockout cell lysate **ab261674**). Wild-type and S100A4 knockout samples were subjected to SDS-PAGE. Ab218511 and **ab181602** (Rabbit anti GAPDH loading control) were incubated overnight at 4°C at 1 ug/ml and 1/20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed **ab216772** and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed **ab216777** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Next Generation Sequencing - Human S100A4 knockout A549 cell line (ab261865)

Knockout achieved by CRISPR/Cas9; X = 1 bp insertion;
Frameshift = 95%

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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