abcam

Product datasheet

Human S100A4 knockout A549 cell line ab261865

6 图像

概述

产品名称	人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)
经测试应用	**适用于:** WB, Next Generation Sequencing
Biosafety level	1
常规说明	**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.

性能

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
存放说明	Shipped on Dry Ice. Store in liquid nitrogen.
存储溶液	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

靶标

组织特异性	Ubiquitously expressed.
序列相似性	Belongs to the S-100 family. Contains 2 EF-hand domains.

应用

The Abpromise guarantee **Abpromise™承诺保证使用**ab261865**于以下的**经测试应用

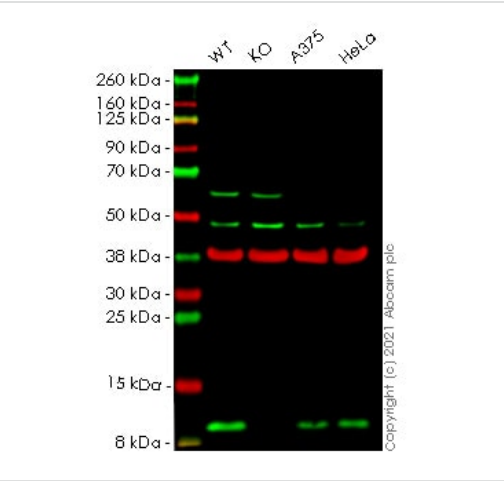
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		Use at an assay dependent concentration.
Next Generation Sequencing		Use at an assay dependent concentration.

图片

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.

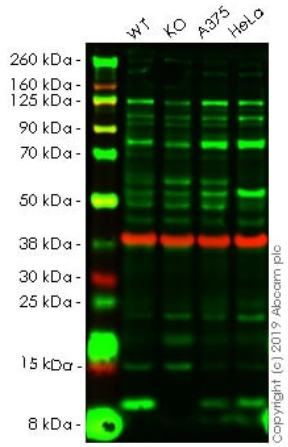
Performed under reducing conditions.

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

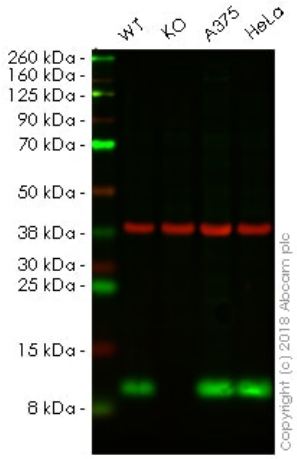
Lysates/proteins at 20 µg per lane.

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

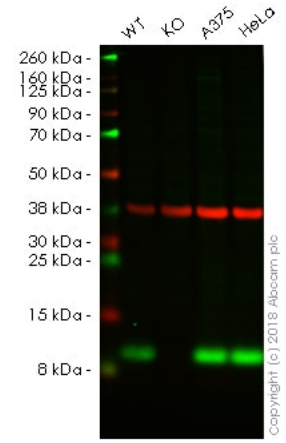
Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 2 : Human S100A4 knockout A549 (Human lung carcinoma cell line) whole cell lysate
Lane 3 : A-375 (Human malignant melanoma cell line) whole cell lysate
Lane 4 : HeLa (Human epithelial cell line from cervix adenocarcinoma)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

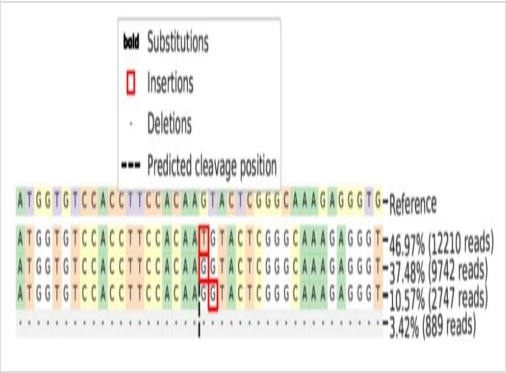
Lysates/proteins at 20 µg per lane.

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%

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