abcam

Product datasheet

Human CD274 (PD-L1) knockout A549 cell line ab267054

画像数 9

製品の概要

製品名	Human CD274 (PD-L1) knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 4 and 2 bp deletion in exon 4 and 7 bp deletion in exon 4
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
アプリケーション	**適用あり:** WB
Biosafety level	2
特記事項	**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 -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.

製品の特性

Number of cells	1 x 10^6 cells/vial, 1 mL
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
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

機能	Involved in the costimulatory signal, essential for T-cell proliferation and production of IL10 and IFNG, in an IL2-dependent and a PDCD1-independent manner. Interaction with PDCD1 inhibits T-cell proliferation and cytokine production.
組織特異性	Highly expressed in the heart, skeletal muscle, placenta and lung. Weakly expressed in the thymus, spleen, kidney and liver. Expressed on activated T- and B-cells, dendritic cells, keratinocytes and monocytes.
配列類似性	Belongs to the immunoglobulin superfamily. BTN/MOG family. Contains 1 Ig-like C2-type (immunoglobulin-like) domain. Contains 1 Ig-like V-type (immunoglobulin-like) domain.
細胞内局在	Cell membrane and Endomembrane system.

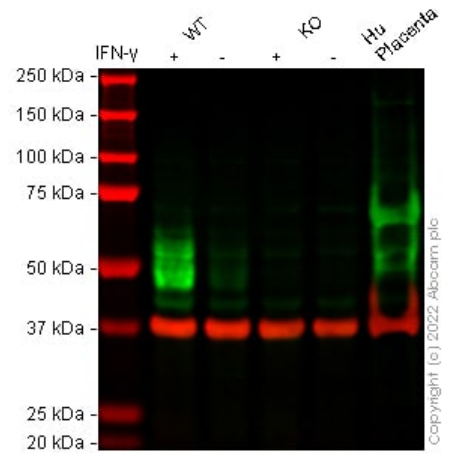
アプリケーション

The Abpromise guarantee	**Abpromise保証は、次のテスト済みアプリケーションにおけるab267054の使用に適用されます**

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 33 kDa.

画像



Western blot - Human CD274 (PD-L1) knockout A549 cell line (ab267054)

All lanes : Anti-PD-L1 antibody [CAL10] - Mouse IgG2a (Chimeric) (**ab279293**) at 1/1000 dilution

Lane 1 : Wild-type A549 Treated IFN-gamma (100 ng/mL, 48 h) cell lysate
Lane 2 : Wild-type A549 Vehicle Control IFN-gamma (0 ng/mL, 48 h) cell lysate
Lane 3 : CD274 knockout A549 Treated IFN-gamma (100 ng/mL, 48 h) cell lysate
Lane 4 : CD274 knockout A549 Vehicle Control IFN-gamma (0 ng/mL, 48 h) cell lysate
Lane 5 : Human Placenta cell lysate

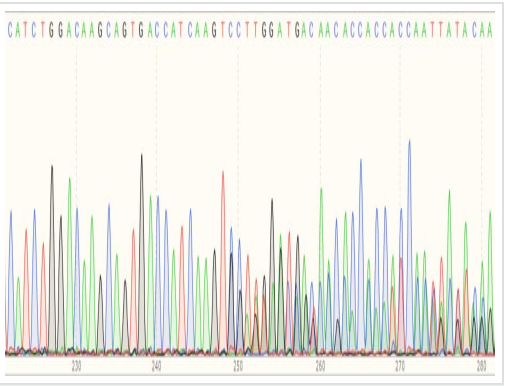
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 33 kDa
Observed band size: 45-65 kDa

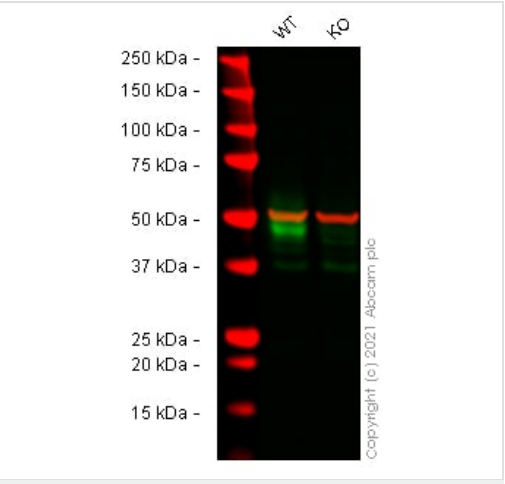
False colour image of Western blot: Anti-PD-L1 antibody [CAL10] - Mouse IgG2a staining at 1/1000 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (**ab181602**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab279293** was shown to bind specifically to PD-L1. A band was observed at 45-65 kDa in treated wild-type A549 cell lysates with no signal observed at this size in Cd274 knockout cell line ab267054 (knockout cell lysate **ab256831**). To generate this image, wild-type and Cd274 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 fluorescent western blot (TBS-based) blocking solution 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.



Sanger Sequencing - Human CD274 (PD-L1) knockout A549 cell line (ab267054)

Sequencing chromatogram displaying sequence edit in exon 4



Western blot - Human CD274 (PD-L1) knockout A549 cell line (ab267054)

All lanes : Anti-PD-L1 antibody [CAL10] - Rat IgG2a (Chimeric) (**ab279294**) at 1/1000 dilution

Lane 1 : Wild-type A549 Treated IFN-gamma (100 ng/ml) for 48 hours cell lysate
Lane 2 : CD274 knockout A549 Treated IFN-gamma (100 ng/ml) for 48 hours cell lysate

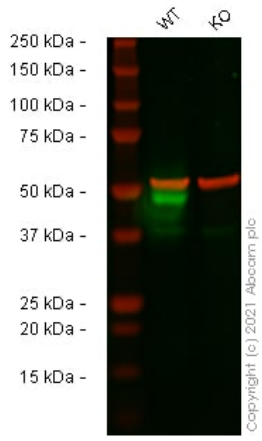
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 33 kDa
Observed band size: 48 kDa

False colour image of Western blot: Anti-PD-L1 antibody [CAL10] - Rat IgG2a staining at 1/1000 dilution, shown in green; Rabbit anti-alpha Tubulin antibody [EP1332Y] (**ab52866**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab279294** was shown to bind specifically to PD-L1. A band was observed at 48 kDa in treated wild-type A549 cell lysates with no signal observed at this size in Cd274 knockout cell line ab267054 (knockout cell lysate **ab256831**). To generate this image, wild-type and Cd274 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-Rat IgG H&L (IRDye® 800CW) preabsorbed (**ab253031**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) at 1/20000 dilution.



Western blot - Human CD274 (PD-L1) knockout A549 cell line (ab267054)

All lanes : Anti-PD-L1 antibody [CAL10] - Mouse IgG1 (Chimeric) (**ab279292**) at 1/1000 dilution

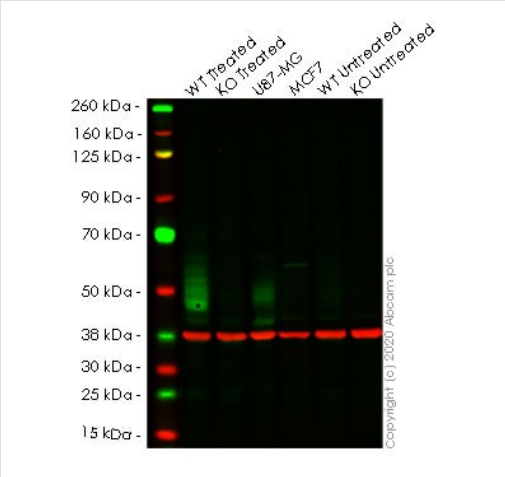
Lane 1 : Wild-type A549 Treated IFN-gamma (100 ng/ml) for 48 hours cell lysate
Lane 2 : CD274 knockout A549 Treated IFN-gamma (100 ng/ml) for 48 hours cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 33 kDa
Observed band size: 48 kDa

False colour image of Western blot: Anti-PD-L1 antibody [CAL10] - Mouse IgG1 staining at 1/1000 dilution, shown in green; Rabbit anti-alpha Tubulin antibody [EP1332Y] (**ab52866**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab279292** was shown to bind specifically to PD-L1. A band was observed at 48 kDa in treated wild-type A549 cell lysates with no signal observed at this size in Cd274 knockout cell line ab267054 (knockout cell lysate **ab256831**). To generate this image, wild-type and Cd274 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-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) at 1/20000 dilution.



Western blot - Human CD274 (PD-L1) knockout A549 cell line (ab267054)

All lanes : Anti-PD-L1 antibody [EPR19759] (**ab213524**) at 1/1000 dilution

Lane 1 : Wild-type A549 treated with 100 ng/ml IFN gamma (**ab259377**) for 48 h cell lysate
Lane 2 : CD274 knockout A549 treated with 100 ng/ml IFN gamma (**ab259377**) for 48 h cell lysate
Lane 3 : U-87 MG cell lysate
Lane 4 : MCF7 cell lysate
Lane 5 : Wild-type A549 untreated cell lysate
Lane 6 : CD274 knockout A549 untreated cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 33 kDa
Observed band size: 50 kDa

Lanes 1- 6: Merged signal (red and green). Green - **ab213524** observed at 50 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab213524 was shown to react with PD-L1 in wild-type A549 treated with 100 ng/ml IFN gamma for 48 h cells in western blot. Loss of signal was observed when both treated and untreated knockout cell lines ab267054 (treated and untreated knockout cell lysates **ab256831**) were used. Wild-type A549 treated with 100 ng/ml IFN gamma for 48 h and CD274 knockout A549 treated with 100 ng/ml IFN gamma for 48 h cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab213524** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 1000 dilution and a 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 CD274 knockout A549 cell line (ab267054)

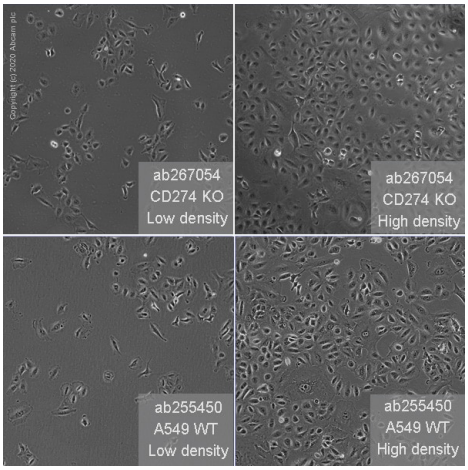
Allele-1: 7 bp deletion in exon4

Sanger Sequencing - Human CD274 knockout A549 cell line (ab267054)

Allele-2: 2 bp deletion in exon 4.

Sanger Sequencing - Human CD274 knockout A549 cell line (ab267054)

Allele-3: 1 bp insertion in exon 4.



Cell Culture - Human CD274 (PD-L1) knockout A549 cell line (ab267054)

Representative images of CD274 knockout A549 cells, low and high confluency examples (top left and right respectively) and wild-type A549 cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using an EVOS M5000 microscope.

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