abcam

Product datasheet

Human IL-6 knockout A549 cell line ab273751

画像数 5

製品の概要

製品名	Human IL-6 knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 97 bp deletion in exon 3
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
アプリケーション	**適用あり:** WB, Sandwich ELISA
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
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

機能	Cytokine with a wide variety of biological functions. It is a potent inducer of the acute phase response. Plays an essential role in the final differentiation of B-cells into Ig-secreting cells Involved in lymphocyte and monocyte differentiation. It induces myeloma and plasmacytoma growth and induces nerve cells differentiation Acts on B-cells, T-cells, hepatocytes, hematopoeitic progenitor cells and cells of the CNS. Also acts as a myokine. It is discharged into the bloodstream after muscle contraction and acts to increase the breakdown of fats and to improve insulin resistance.
関連疾患	Genetic variations in IL6 are associated with susceptibility to rheumatoid arthritis systemic juvenile (RASJ) [MIM:604302]. An inflammatory articular disorder with systemic-onset beginning before the age of 16. It represents a subgroup of juvenile arthritis associated with severe extraarticular features and occasionally fatal complications. During active phases of the disorder, patients display a typical daily spiking fever, an evanescent macular rash, lymphadenopathy, hepatosplenomegaly, serositis, myalgia and arthritis. Note=A IL6 promoter polymorphism is associated with a lifetime risk of development of Kaposi sarcoma in HIV-infected men.
配列類似性	Belongs to the IL-6 superfamily.
翻訳後修飾	N- and O-glycosylated.
細胞内局在	Secreted.

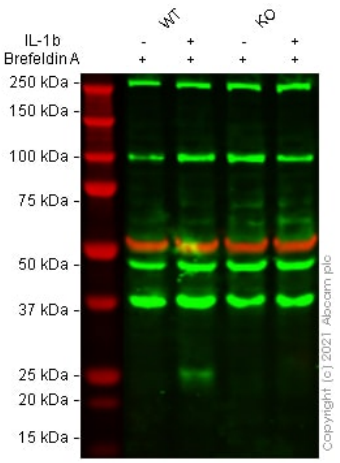
アプリケーション

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ab273751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: 23 kDa.
Sandwich ELISA		Use at an assay dependent concentration.

画像



Western blot - Human IL-6 knockout A549 cell line (ab273751)

All lanes : Anti-IL-6 antibody [EPR22565-204] (**ab233551**) at 1/1000 dilution

Lane 1 : Wild-type A549 Vehicle Control IL-1b (0 ng/mL, 24 h), Brefeldin A (5 ug/mL, final 6 h) cell lysate
Lane 2 : Wild-type A549 Treated IL-1b (20 ng/mL, 24 h), Brefeldin A (5 ug/mL, final 6 h) cell lysate
Lane 3 : IL-6 knockout A549 Vehicle Control IL-1b (0 ng/mL, 24 h), Brefeldin A (5 ug/mL, final 6 h) cell lysate
Lane 4 : IL-6 knockout A549 Treated IL-1b (20 ng/mL, 24 h), Brefeldin A (5 ug/mL, final 6 h) cell lysate

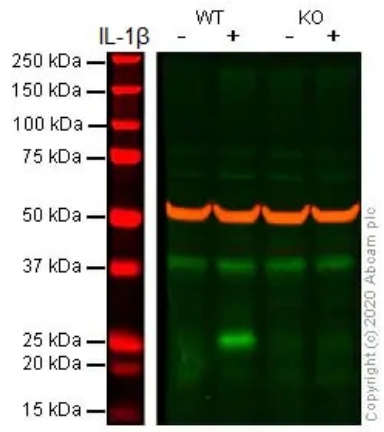
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 23 kDa
Observed band size: 25 kDa

False colour image of Western blot: Anti-IL-6 antibody [EPR22565-204] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab233551** was shown to bind specifically to IL-6. A band was observed at 25 kDa in wild-type A549 cell lysates with no signal observed at this size in IL6 knockout cell line ab273751 (knockout cell lysate **ab275501**). To generate this image, wild-type and IL6 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-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Human IL-6 knockout A549 cell line (ab273751)

All lanes : Anti-IL-6 antibody [EPR21711] (**ab233706**) at 1/1000 dilution

Lane 1 : Wild-type A549 Brefeldin A (**ab120299**)-treated (5ug/ml, 4h) cell lysate
Lane 2 : Wild-type A549 IL-1ß (**ab259387**) (20 ng/ml, 24h) and Brefeldin A (**ab120299**)-treated (5 ug/ml for the last 4h) cell lysate
Lane 3 : IL-6 knockout A549 Brefeldin A (**ab120299**)-treated (5ug/ml, 4h) cell lysate
Lane 4 : IL-6 knockout A549 IL-1ß (**ab259387**) (20 ng/ml, 24h) and Brefeldin A (**ab120299**)-treated (5 ug/ml for the last 4h) cell lysate

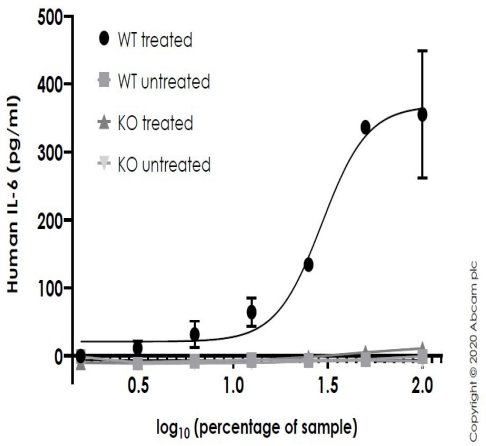
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 23 kDa
Observed band size: 25 kDa
Additional bands at: 40 kDa (possible non-specific binding)

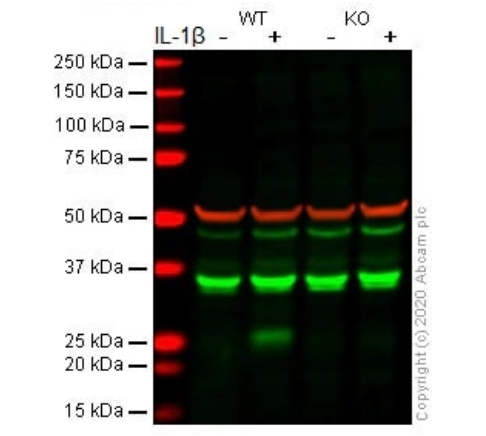
Lanes 1 - 4: Merged signal (red and green). Green - **ab233706** observed at 25 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

ab233706 was shown to react with IL-6 in wild-type A549 cells in western blot with loss of signal observed in IL-6 knockout cell line ab273751 (knockout cell lysate **ab275501**). Wild-type and IL-6 knockout A549 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab233706** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A] 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 hour at room temperature before imaging.



Sandwich ELISA - Human IL-6 knockout A549 cell line (ab273751)

Human IL-6 concentration was interpolated from the IL-6 standard curve. Supernatants from cell culture samples were serially diluted and assessed by the Human IL-6 ELISA kit (**ab178013**). Wild-type and IL-6 knockout A549 cells (ab273751) were assessed in duplicate (n=2). Cells were either treated with 20 ng/mL active recombinant human IL-1 beta protein (**ab259387**) for 24 h to induce expression of IL-6 or not treated with IL-1 beta. Data are represented as the mean and error bars represent standard deviation.



Western blot - Human IL-6 knockout A549 cell line (ab273751)

All lanes : Anti-IL-6 antibody [EPR20653] (**ab214429**) at 1/1000 dilution

Lane 1 : Wild-type A549 Brefeldin A (**ab120299**)-treated (5ug/ml, 4h) cell lysate
Lane 2 : Wild-type A549 IL-1ß (**ab259387**) (20 ng/ml, 24h) and Brefeldin A (**ab120299**)-treated (5 ug/ml for the last 4h) cell lysate
Lane 3 : IL-6 knockout A549 Brefeldin A (**ab120299**)-treated (5ug/ml, 4h) cell lysate
Lane 4 : IL-6 knockout A549 IL-1ß (**ab259387**) (20 ng/ml, 24h) and Brefeldin A (**ab120299**)-treated (5 ug/ml for the last 4h) cell lysate

Lysates/proteins at 30 µg per lane.

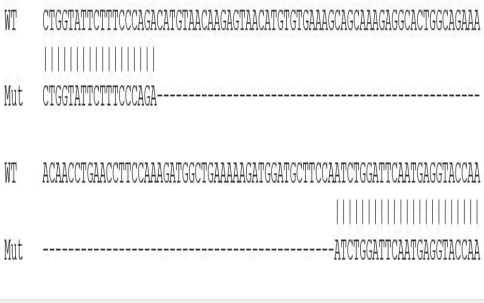
Performed under reducing conditions.

Predicted band size: 23 kDa
Observed band size: 25 kDa
Additional bands at: 35 kDa (possible non-specific binding)

Lanes 1 - 4: Merged signal (red and green). Green - **ab214429** observed at 25 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

ab214429 was shown to react with IL-6 in wild-type A549 cells in western blot with loss of signal observed in IL-6 knockout cell line ab273751 (knockout cell lysate **ab275501**). Wild-type A549 and IL-6 knockout cell lysates were subjected to SDS-PAGE. Membranes

were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab214429** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A] 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 hour at room temperature before imaging.



Sanger Sequencing - Human IL6 knockout A549 cell line (ab273751)

Allele-1: 97 bp deletion in exon 3.

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