abcam

Product datasheet

Human CXCL10 (IP10) knockout A549 cell line ab266971

6 Images

Overview

Product name	Human CXCL10 (IP10) knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 2 and 4 bp deletion in exon 2
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** ICC/IF, WB
Biosafety level	1
General notes	**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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Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Viability	~80%
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
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

Function	Chemotactic for monocytes and T-lymphocytes. Binds to CXCR3.
Sequence similarities	Belongs to the intercrine alpha (chemokine CxC) family.
Post-translational modifications	CXCL10(1-73) is produced by proteolytic cleavage after secretion from keratinocytes.
Cellular localization	Secreted.

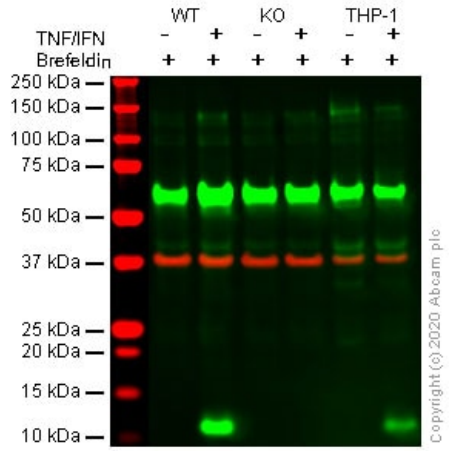
Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab266971 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
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 10 kDa.

Images



Western blot - Human CXCL10 (IP10) knockout A549 cell line (ab266971)

All lanes : Anti-IP10 antibody [EPR20764] (ab214668) at 1/1000 dilution

Lane 1 : Wild-type A549 Brefeldin A (ab120299)-treated (5ug/ml, 6h) cell lysate
Lane 2 : Wild-type A549 IFN-y (ab259377) (100 ng/ml, 32 h) and TNF-alpha (ab259410) (10 ng/ml, 32h), and Brefeldin A (ab120299)-treated (5ug/ml for the last 6h) cell lysate
Lane 3 : IP10 knockout A549 Brefeldin A (ab120299)-treated (5ug/ml, 6h) cell lysate
Lane 4 : IP10 knockout A549 IFN-y (ab259377) (100ng/ml, 32h) and TNF-alpha (ab259410) (10ng/ml, 32h), and Brefeldin A (ab120299)-treated (5ug/ml for the last 6h) cell lysate
Lane 5 : THP-1 Brefeldin A (ab120299)-treated (5ug/ml, 6h) cell lysate
Lane 6 : THP-1 IFN-y (ab259377) (200ng/ml, 24h) and LPS (50ng/ml, 24h)-treated for 24 hours, and Brefeldin A (ab120299)-treated (5ug/ml for the last 6h) cell lysate

Lysates/proteins at 30 µg per lane.

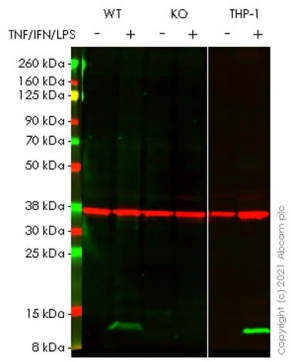
Performed under reducing conditions.

Predicted band size: 10 kDa
Observed band size: 11 kDa

Lanes 1 - 6: Merged signal (red and green). Green - ab214668 observed at 11 kDa. Red - loading control ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab214668 was shown to react with IP10 in wild-type A549 cells in western blot with loss of signal observed in IP10 knockout cell line ab266971 (knockout cell lysate ab256888). Wild-type and IP10 knockout A549 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with ab214668 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® 800CW) preabsorbed (ab216772) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human CXCL10 (IP10) knockout A549 cell line (ab266971)

All lanes : Anti-IP10 antibody [EPR24674-12] (ab283681) at 1/1000 dilution

Lane 1 : Untreated Wild-type A549 (human lung carcinoma epithelial cell), whole cell lysate at 40 µg
Lane 2 : Wild-type A549 treated with 100 ng/ml IFN-y (ab259377) for 32 hours and 10 ng/m TNF-alpha (ab259410) for 32 hours, and 5ug/ml Brefeldin A (ab120299) for the last 6 hours, whole cell lysate at 40 µg
Lane 3 : Untreated IP10 knockout A549 whole cell lysate at 40 µg
Lane 4 : IP10 knockout A549 treated with 100 ng/ml IFN-y (ab259377) for 32 hours and 10 ng/m TNF-alpha (ab259410) for 32 hours, and 5ug/ml Brefeldin A (ab120299) for the last 6 hours, whole cell lysate at 40 µg
Lane 5 : Untreated THP-1 (human monocytic leukemia monocyte), whole cell lysate at 20 µg
Lane 6 : THP-1 treated with 200ng/ml IFN-y (ab259377) for 24 hours and 50ng/ml LPS for 24 hours, and 5ug/ml Brefeldin A for the last 21 hours, whole cell lysate at 20 µg

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (ab216773) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (ab216776) at 1/10000 dilution

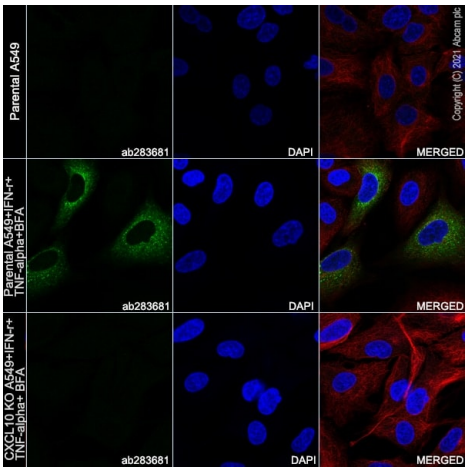
Predicted band size: 10 kDa
Observed band size: 11 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Lanes 1-6: Merged signal (red and green). Green - ab283681 observed at 11 kDa. Red-loading control ab8245 (Mouse monoclonal [6C5] to GAPDH) was observed at 36 kDa.

ab283681 Anti-TNF Receptor I antibody [EPR24674-12] was shown to specifically react with IP10 in treated wild-type A549 cells. Loss of signal was observed when IP10 knockout cell lines ab266971 (knockout cell lysate ab256888) were used. Wild-type and IP10 knockout samples were subjected to SDS-PAGE. ab283681 and Anti-GAPDH antibody [6C5] - Loading Control

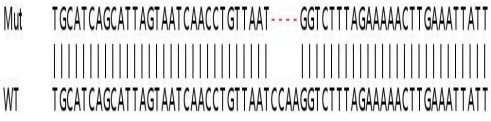
(ab8245) were incubated at 4°C overnight at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Human CXCL10 (IP10) knockout A549 cell line (ab266971)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized CXCL10 KO A549 (ab266971) cells labelling IP10 with ab283681 at 1/50 (12.86 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/ml) dilution (Green). Confocal image showing the signal expression was increased in Parental A549 cells after treatment with Interferon gamma (200 ng/ml) and lipopolysaccharide (50 ng/ml) for 3h, then adding Brefeldin A (1 ug/ml) for another 21h, and no staining in treated CXCL10 KO A549 cells with the same conditions. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5 ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution.

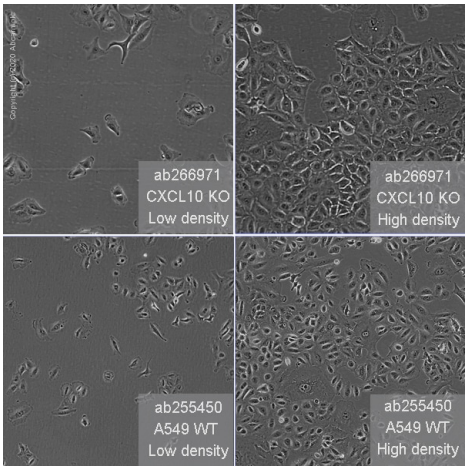


Sanger Sequencing - Human CXCL10 knockout A549 cell line (ab266971)

Allele-1: 4 bp deletion in exon2

Sanger Sequencing - Human CXCL10 knockout A549 cell line (ab266971)

Allele-2: 1 bp insertion in exon 2.



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