abcam

Product datasheet

Human CXCL8 knockout PC-3 cell line ab273743

5 Images

Overview

Product name	Human CXCL8 knockout PC-3 cell line
Parental Cell Line	PC3
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 22% 11 bp deletion, 24% 7 bp deletion, 54% 2 bp deletion in exon 2
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS), Western Blot (WB)
Tested applications	**Suitable for:** WB, Sandwich ELISA
Biosafety level	1
General notes	**Recommended control:** Human wild-type PC3 cell line (**ab275472**). 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 $2x10^4$ 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 $2x10^4$ 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.
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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	Prostate
Cell type	epithelial
Disease	Adenocarcinoma
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

Function	IL-8 is a chemotactic factor that attracts neutrophils, basophils, and T-cells, but not monocytes. It is also involved in neutrophil activation. It is released from several cell types in response to an inflammatory stimulus. IL-8(6-77) has a 5-10-fold higher activity on neutrophil activation, IL-8(5-77) has increased activity on neutrophil activation and IL-8(7-77) has a higher affinity to receptors CXCR1 and CXCR2 as compared to IL-8(1-77), respectively.
Sequence similarities	Belongs to the intercrine alpha (chemokine CxC) family.
Post-translational modifications	Several N-terminal processed forms are produced by proteolytic cleavage after secretion from at least peripheral blood monocytes, leukcocytes and endothelial cells. In general, IL-8(1-77) is referred to as interleukin-8. IL-8(6-77) is the most promiment form.
Cellular localization	Secreted.

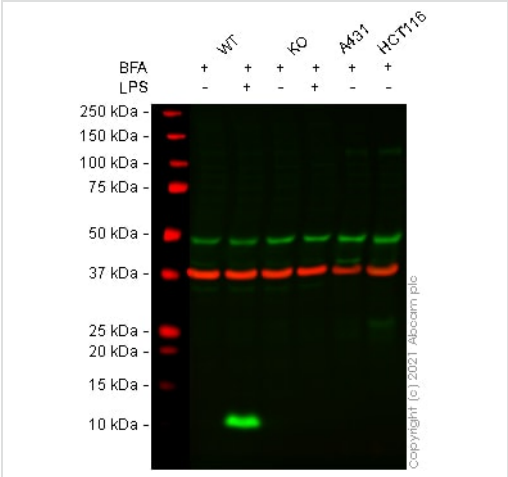
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab273743 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.
Sandwich ELISA		Use at an assay dependent concentration.

Images



Western blot - Human CXCL8 knockout PC3 cell line (ab273743)

All lanes : Anti-IL-8 antibody [EPR22994-255] (**ab235584**) at 1/1000 dilution

Lane 1 : Wild-type PC-3 Brefeldin A (**ab120299**)-treated (5 µg/ml, 5 h) cell lysate
Lane 2 : Wild-type PC-3 LPS-treated (2 µg/ml, 6 h) with Brefeldin A (**ab120299**) (5 µg/ml, 5 h) cell lysate
Lane 3 : CXCL8 knockout PC-3 Brefeldin A (**ab120299**)-treated (5 µg/ml, 5 h) cell lysate
Lane 4 : CXCL8 knockout PC-3 LPS-treated (2 µg/ml, 6 h) with Brefeldin A (**ab120299**) (5 µg/ml, 5 h) cell lysate
Lane 5 : A431 cell lysate
Lane 6 : HCT116 cell lysate

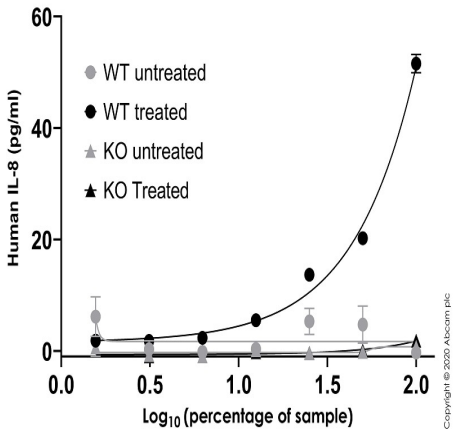
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Observed band size: 10 kDa

Lanes 1 - 6: Merged signal (red and green). Green - **ab235584** observed at 10 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab235584 was shown to react with IL-8 in wild-type PC-3 cells in Western blot with loss of signal observed in CXCL8 knockout cell line ab273743 (knockout cell lysate **ab275520**). Wild-type PC-3 and CXCL8 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab235584** 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.

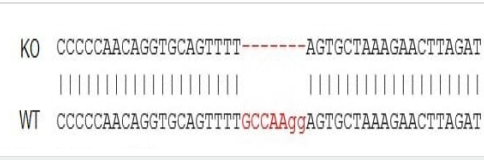


Sandwich ELISA - Human CXCL8 knockout PC-3 cell line (ab273743)

Human IL-8 concentration was interpolated from the standard curve. Supernatants from cell culture samples were serially diluted and assessed by the Human IL-8 ELISA kit (**ab214030**). Wild-type PC-3 cells and CXCL8 knockout PC-3 cells (ab273743) were assessed in duplicate (n=2) and were either treated with 2 µg/ml LPS for 6 hours to induce expression of IL-8 or not treated with LPS. Data are represented as the mean and error bars represent standard deviation.

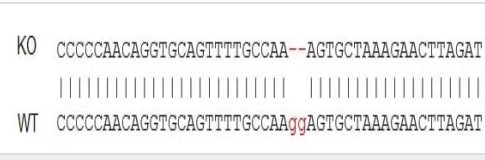
Next Generation Sequencing - Human CXCL8 (IL-8) knockout PC3 cell line (ab273743)

Allele-1: 11bp deletion in exon 2.



Next Generation Sequencing - Human CXCL8 (IL-8) knockout PC3 cell line (ab273743)

Allele-2: 7bp deletion in exon 2.



Next Generation Sequencing - Human CXCL8 (IL-8) knockout PC3 cell line (ab273743)

Allele-3: 2bp deletion in exon 2.

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