

Human CXCL8 knockout PC-3 cell lysate ab275520

4 Images

Overview

Product name	Human CXCL8 knockout PC-3 cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9. Treatments: Human CXCL8 knockout PC3 cell lysate - Brefeldin A (5 µg/ml, 5h) Human wild-type PC3 cell lysate - Brefeldin A (5 µg/ml, 5h) Human CXCL8 knockout PC3 cell lysate - LPS (2 µg/ml, 6h) and Brefeldin A (5 µg/ml, 5h) Human wild-type PC3 cell lysate - LPS (2 µg/ml, 6h) and Brefeldin A (5 µg/ml, 5h)
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)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. <i>*Usage of SDS sample buffer is not recommended with these lyophilized lysates.</i>

Notes

Lysate preparation: Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found [here](#). Please refer to our lysis protocol for further details on how our lysates are prepared.

User storage instructions: Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage.

Access thousands of knockout cell lysates, generated from commonly used cancer cell lines.

[See here for more information on knockout cell lysates.](#)

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It is the responsibility of our customers to check the necessity of application of REACH

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Tested applications

Suitable for: WB

Properties

Storage instructions Store at -80°C. Please refer to protocols.

Components	1 kit
ab277344 - Human CXCL8 knockout PC3 cell lysate - Brefeldin A treated	1 x 100µg
ab277343 - Human CXCL8 knockout PC3 cell lysate - LPS + Brefeldin A treated	1 x 100µg
ab277346 - Human wild-type PC3 cell lysate - Brefeldin A treated	1 x 100µg
ab277345 - Human wild-type PC3 cell lysate - LPS + Brefeldin A treated	1 x 100µg

Cell type epithelial
Disease Adenocarcinoma
Gender Male

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, leukocytes and endothelial cells. In general, IL-8(1-77) is referred to as interleukin-8. IL-8(6-77) is the most prominent form.

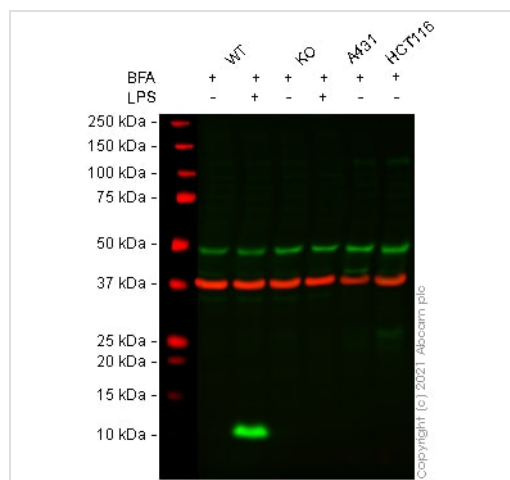
Cellular localization Secreted.

Applications

The Abpromise guarantee Our [Abpromise guarantee](#) covers the use of ab275520 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.

Images



Western blot - Human CXCL8 knockout PC3 cell lysate (ab275520)

Lane 1: Wild-type PC-3 Brefeldin A ([ab120299](#))-treated (5 µg/ml, 5 h) cell lysate 30 µg

Lane 2: Wild-type PC-3 LPS-treated (2 µg/ml, 6 h) with Brefeldin A ([ab120299](#)) (5 µg/ml, 5 h) cell lysate 30 µg

Lane 3: CXCL8 knockout PC-3 Brefeldin A ([ab120299](#))-treated (5 µg/ml, 5 h) cell lysate 30 µg

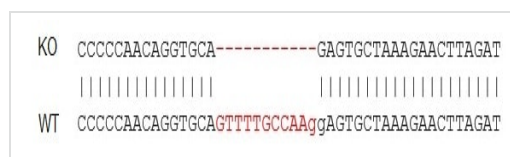
Lane 4: CXCL8 knockout PC-3 LPS-treated (2 µg/ml, 6 h) with Brefeldin A ([ab120299](#)) (5 µg/ml, 5 h) cell lysate 30 µg

Lane 5: A431 cell lysate 30 µg

Lane 6: HCT116 cell lysate 30 µg

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 37kDa.

[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.



Next Generation Sequencing - Human CXCL8 knockout PC3 cell lysate

Allele-1: 11bp deletion in exon 2.

```

KO  CCCCCAACAGGTGCAGTTTT-----AGTGCTAAAGAACTTAGAT
    |||
WT  CCCCCAACAGGTGCAGTTTTGCCAAggAGTGCTAAAGAACTTAGAT

```

Next Generation Sequencing - Human CXCL8
knockout PC3 cell lysate

Allele-2: 7bp deletion in exon 2.

```

KO  CCCCCAACAGGTGCAGTTTTGCCAA--AGTGCTAAAGAACTTAGAT
    |||
WT  CCCCCAACAGGTGCAGTTTTGCCAAggAGTGCTAAAGAACTTAGAT

```

Next Generation Sequencing - Human CXCL8
knockout PC3 cell lysate

Allele-3: 2bp deletion in exon 2.

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