abcam

ab289967

Anti-IL-8 antibody [EPR26511-74]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR26511-74
Purification technique	Affinity purification Protein A
Concentration	0.455 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Human
Dilution info	1/100
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/500
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

Target data

See full target information CXCL8

Function	Chemotactic factor that mediates inflammatory response by attracting neutrophils, basophils, and T-cells to clear pathogens and protect the host from infection (PubMed:18692776, PubMed:7636208). Also plays an important role in neutrophil activation (PubMed:2145175, PubMed:9623510). Released in response to an inflammatory stimulus, exerts its effect by binding to the G-protein-coupled receptors CXCR1 and CXCR2, primarily found in neutrophils, monocytes and endothelial cells (PubMed:1840701, PubMed:1891716). G-protein heterotrimer (alpha, beta, gamma subunits) constitutively binds to CXCR1/CXCR2 receptor and activation by IL8 leads to beta and gamma subunits release from Galpha (GNAI2 in neutrophils) and activation of several downstream signaling pathways including PI3K and MAPK pathways (PubMed:11971003, PubMed:8662698).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks

Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	IL-8 also known as CXCL8 is a cytokine with a molecular weight of approximately 8 kDa. It belongs to the CXC chemokine family and plays a role in the recruitment of neutrophils to sites of inflammation. Cells like monocytes macrophages and endothelial cells express IL-8 after activation by pro-inflammatory signals. The protein exhibits high expression in cells associated with the immune response and inflammation serving as a signaling molecule between these cell types.
Biological function summary	IL-8 functions as a chemoattractant for neutrophils and lymphocytes facilitating their movement towards the site of infection or injury. It does not form part of a larger protein complex but operates individually to enhance immune cell migration. IL-8 possesses unique binding motifs allowing it to interact with specific receptors namely CXCR1 and CXCR2 on target cells. This binding triggers cellular responses leading to effective immune surveillance and response to inflammatory stimuli.
Pathways	IL-8 operates within important inflammatory and immune response pathways. It forms a part of the NF-κB signaling cascade which activates in response to stress signals promoting the expression of other inflammatory mediators. Additionally it engages in the mitogen-activated protein kinase (MAPK) pathway influencing cellular responses such as proliferation and differentiation. The interaction of IL-8

	with these pathways highlights its role in modulating immune responses and highlights its interaction with other proteins such as TNF-α and IL-1β.
Associated diseases and disorders	IL-8 shows a strong association with conditions like rheumatoid arthritis and chronic obstructive pulmonary disease (COPD). It acts as a biomarker for these diseases often correlating with disease severity and inflammation levels. In rheumatoid arthritis IL-8 contributes to joint inflammation and degradation while in COPD it enhances airway inflammation and tissue damage. The cytokine's involvement in these diseases connects it with other inflammatory mediators such as MMPs and leukotrienes which collectively exacerbate disease pathology.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

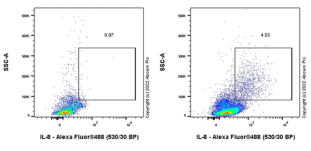
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

6 product images

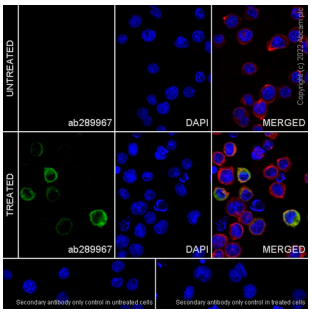


Flow Cytometry (Intracellular) - Anti-IL-8 antibody [EPR26511-74] (ab289967)

Intracellular flow cytometric analysis of 2% paraformaldehyde fixed, 0.1% saponin permeabilised human peripheral blood mononuclear cell (PBMC) treated with 1µg/ml Lipopolysaccharide (LPS) for 22 hours, then add 3uM Monensin for another 2h (Right). Untreated control (Left).
Primary antibody: ab289967, at 1/500 dilution.

Secondary antibody: Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution.

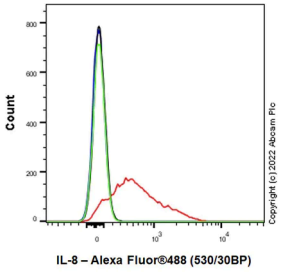
Scatter image shows specific IL-8 expression in LPS induced monocyte population.



Immunocytochemistry/ Immunofluorescence - Anti-IL-8 antibody [EPR26511-74] (ab289967)

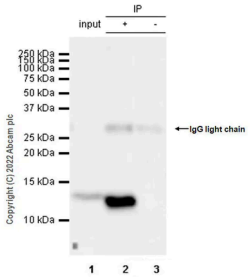
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized U-937 cells labelling IL-8 with ab289967 at 1/100 (5.87 µg/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 µg/mL) dilution (Green). Confocal image showing cytoplasmic staining is observed in U-937 cells treated with TPA (100 ng/mL) for 24 h, then LPS (5 µg/mL) for 7 h with Brefeldin A (300 ng/mL) for the last 3 h. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5µg/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 µg/mL) dilution.



Flow Cytometry (Intracellular) - Anti-IL-8 antibody [EPR26511-74] (ab289967)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized U-937 (Human histiocytic lymphoma monocyte) treated with 100ng/ml TPA for 24 hours, then 5µg/ml LPS for 4 hours, and add 300ng/ml BFA for another 3h (Red) / Untreated control (Green) cells labelling IL-8 with ab289967 at 1/500 dilution (0.1µg) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-IL-8 antibody [EPR26511-74] (ab289967)

IL-8 was immunoprecipitated from U937 (human histiocytic lymphoma monocyte) treated with 100ng/ml TPA for 24h then treated with 5 µg/ml LPS for 4h, and add 300 ng/ml BFA for another 3h, whole cell lysate with ab289967 at 1/30 dilution (2 µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab289967 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.
Lane 1: U937 (human histiocytic lymphoma monocyte) treated with 100 ng/ml TPA for 24h then treated with 5 µg/ml LPS for 4h, and add 300 ng/ml BFA for another 3h, whole cell lysate 10 µg

Lane 2: ab289967 IP in U937 treated with 100 ng/ml TPA for 24h then treated with 5 µg/ml LPS for 4h, and add 300 ng/ml BFA for another 3h, whole cell lysate

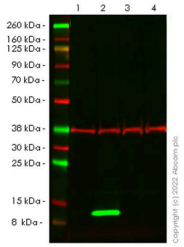
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab289967 in U937 treated with 100 ng/ml TPA for 24h then treated with 5 µg/ml LPS for 4h, and add 300 ng/ml BFA for another 3h, whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 50 seconds

All lanes:
Immunoprecipitation - Anti-IL-8 antibody [EPR26511-74] (ab289967)

Predicted band size: 11 kDa



Western blot - Anti-IL-8 antibody [EPR26511-74] (ab289967)

Blocking buffer and concentration was Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS.
Diluting buffer was Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBST.

Lysates/proteins at 20 µg per lane.
Performed under reducing conditions.
False colour image of Western blot: Anti-IL-8 antibody [EPR26511-74] (ab289967) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red.
In Western blot, ab289967 was shown to bind specifically to IL-8. A band was observed at 11 kDa in wild-type PC-3 cell lysates with no signal observed at this size in CXCL8 knockout cell lysates. To generate this image, wild-type and CXCL8 knockout PC-3 cell lysates were analyzed. First, samples were run on an SDS-PAGE gel then transferred onto an immobilon-FL PVDF membrane. Membranes were blocked in Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS 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 1/10000 dilution.

All lanes:
Western blot - Anti-IL-8 antibody [EPR26511-74] (ab289967) at 1/1000 dilution

Lane 1:
Wild-type PC-3 (human prostate adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
Wild-type PC-3 treated with 2µg/ml LPS for 5h, then treated with 5µg/ml Brefeldin A for 5h, whole cell lysate at 20 µg

Lane 3:
CXCL8 knockout PC-3 whole cell lysate at 20 µg

Lane 4:
CXCL8 knockout PC-3 treated with 2µg/ml LPS for 5h, then treated with 5µg/ml Brefeldin A for 5h, whole cell lysate at 20 µg

Secondary

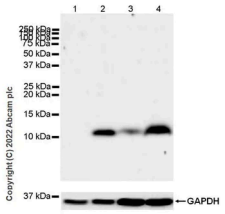
Lanes 1 - 4:
Western blot - Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) at 1/10000 dilution

Lanes 1 - 4:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 11 kDa

Observed band size: 11 kDa

Western blot - Anti-IL-8 antibody [EPR26511-74] (ab289967)



Blocking and diluting buffer and concentration was 5% NFDM/TBST.
All lanes:
Western blot - Anti-IL-8 antibody [EPR26511-74] (ab289967) at 1/1000 dilution

Lane 1:
Untreated U-937 (human histiocytic lymphoma monocyte) whole cell lysate at 20 µg

Lane 2:
U-937 treated with TPA (100ng/mL) for 24 h, then treated with LPS (5 µg/mL) for 7 h with Brefeldin A (300 ng/mL) for the last 3 h, whole cell lysate at 20 µg

Lane 3:
Untreated U-87 MG (human glioblastoma-astrocytoma epithelial cell) whole cell lysate at 20 µg

Lane 4:
U-87 MG treated with 1µM Thapsigargin for 24h, whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 11 kDa

Observed band size: 11 kDa

Exposure time: 70s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.