abcam

Product datasheet

Anti-IL-8 antibody [EPR26511-74] ab289967

KO VALIDATED Recombinant RabMAb

1 References 7 Images

Overview

Product name	Anti-IL-8 antibody [EPR26511-74]
Description	Rabbit monoclonal [EPR26511-74] to IL-8
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, Flow Cyt (Intra), IP, WB **Unsuitable for:** IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Wild-type treated PC-3, treated U-937, Untreated U-87 MG, U-87 MG treated with 1µM Thapsigargin for 24h. ICC/IF: Treated U-937 cells. Flow Cyt (intra): Treated U-937 cells, treated Human peripheral blood mononuclear cell (PBMC). IP: treated U-937 whole cell lysate
General notes	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 production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR26511-74

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab289967 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		1/100.
Flow Cyt (Intra)		1/500.
IP		1/30.
WB		1/1000. Predicted molecular weight: 11 kDa.

Application notes Is unsuitable for IHC-P.

Target

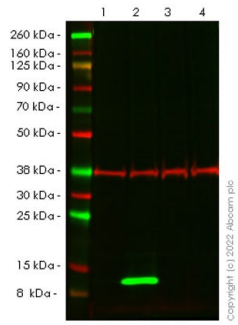
Function IL-8 is a chemotactic factor that attracts neutrophils, basophils, and T-cells, but not monocytes. It is also involved in neutrophil activation. It is released from several cell types in response to an inflammatory stimulus. IL-8(6-77) has a 5-10-fold higher activity on neutrophil activation, 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.

Images



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

All lanes : 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
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
Lane 3 : CXCL8 knockout PC-3 whole cell lysate
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

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) at 1/10000 dilution

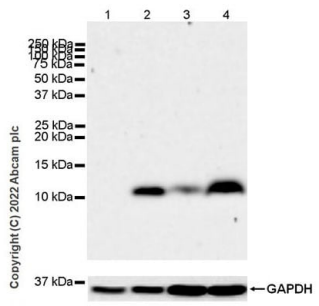
Predicted band size: 11 kDa
Observed band size: 11 kDa

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.



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

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

Lane 1 : Untreated U-937 (human histiocytic lymphoma monocyte) whole cell lysate
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
Lane 3 : Untreated U-87 MG (human glioblastoma-astrocytoma epithelial cell) whole cell lysate
Lane 4 : U-87 MG treated with 1µM Thapsigargin for 24h, whole cell lysate

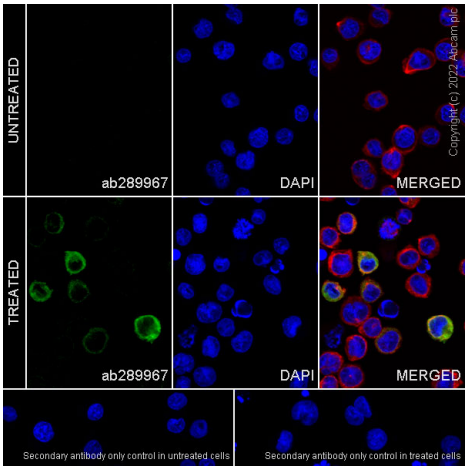
Lysates/proteins at 20 µg per lane.

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

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

Exposure time: 70 seconds

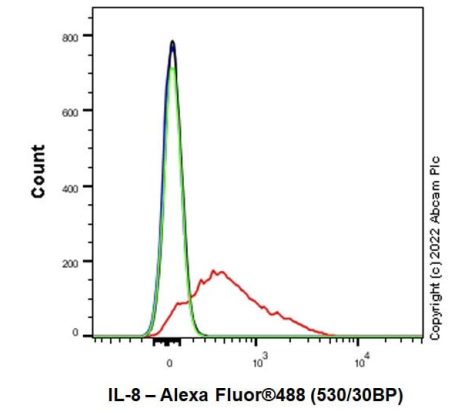
Blocking and diluting buffer and concentration was 5% NFDM/TBST.



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

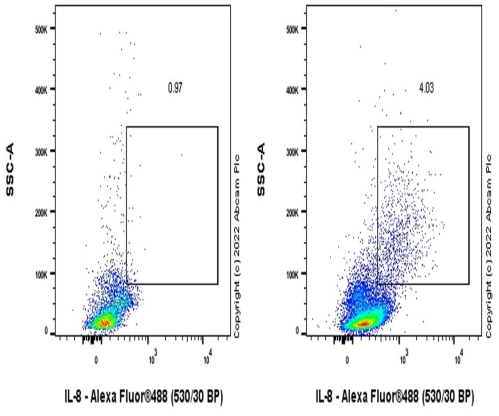
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized U-937 cells labelling IL-8 with ab289967 at 1/100 (5.87 µg/ml) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 µg/mL) dilution (Green). Confocal image 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.



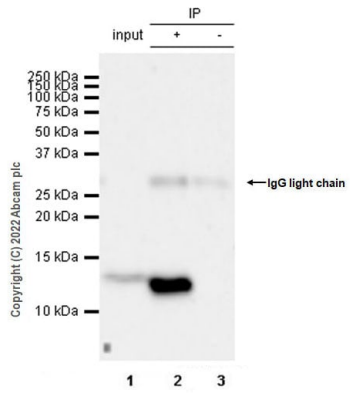
Flow Cytometry (Intracellular) - Anti-IL-8 antibody [EPR26511-74] (ab289967)

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.



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

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

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