abcam

Product datasheet

Anti-CXCL5 + CXCL6 antibody [EPR22310-196] ab243097

KO VALIDATED Recombinant RabMAb

6 Images

Overview

Product name	Anti-CXCL5 + CXCL6 antibody [EPR22310-196]
Description	Rabbit monoclonal [EPR22310-196] to CXCL5 + CXCL6
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB, Flow Cyt (Intra) **Unsuitable for:** IHC-P or IP
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: His-GST-tagged human CXCL5 recombinant protein (aa37-114); Human CXCL6 recombinant protein (aa40-114) without tag; A549 cells starved overnight, treated with 10 ng/ml TNF-a, 10nM PMA and 0.1% BSA (24 h) cell lysate; Wild-type A549 Treated TNFa (10 ng/mL, 24 h) + PMA (10 nM, 24 h) cell lysate. ICC/IF: A549 cells treated with TNF alpha (10ng/ml 24h), PMA (10nM 24h) and BSA (0.1% 24h). Flow Cyt (intra): A549 cells treated with TNF alpha (10ng/ml 24h), PMA (10nM 24h) and BSA (0.1% 24h).
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: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR22310-196
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab243097 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		1/1000.
WB		1/1000. Detects a band of approximately 12 kDa (predicted molecular weight: 12 kDa).
Flow Cyt (Intra)		1/500.

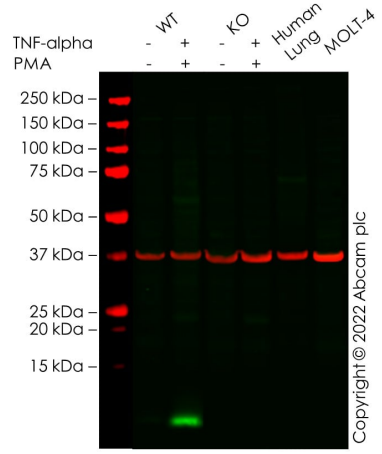
Application notes Is unsuitable for IHC-P or IP.

Target

Relevance CXCL5: Involved in neutrophil activation. In vitro, ENA-78(8-78) and ENA-78(9-78) show a threefold higher chemotactic activity for neutrophil granulocytes. CXCL6: Chemotactic for neutrophil granulocytes. Signals through binding and activation of its receptors (CXCR1 and CXCR2). In addition to its chemotactic and angiogenic properties, it has strong antibacterial activity against Gram-positive and Gram-negative bacteria (90-fold-higher when compared to CXCL5 and CXCL7).

Cellular localization Secreted

Images



Western blot - Anti-CXCL5 + CXCL6 antibody [EPR22310-196] (ab243097)

All lanes : Anti-CXCL5 + CXCL6 antibody [EPR22310-196] (ab243097) at 1/1000 dilution

Lane 1 : Wild-type A549 Untreated Control cell lysate
Lane 2 : Wild-type A549 Treated TNFa (10 ng/mL, 24 h) + PMA (10 nM, 24 h) cell lysate
Lane 3 : CXCL5 knockout A549 Untreated Control cell lysate
Lane 4 : CXCL5 knockout A549 Treated TNFa (10 ng/mL, 24 h) + PMA (10 nM, 24 h) cell lysate
Lane 5 : Human Lung cell lysate
Lane 6 : MOLT-4 cell lysate

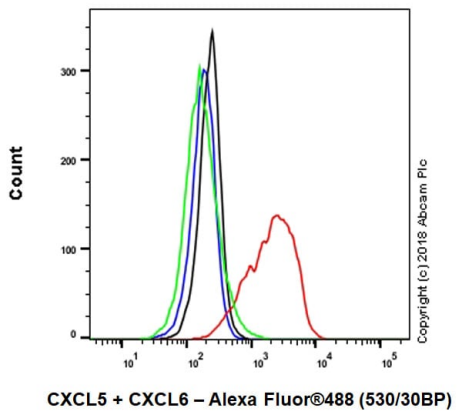
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 12 kDa
Observed band size: 12 kDa

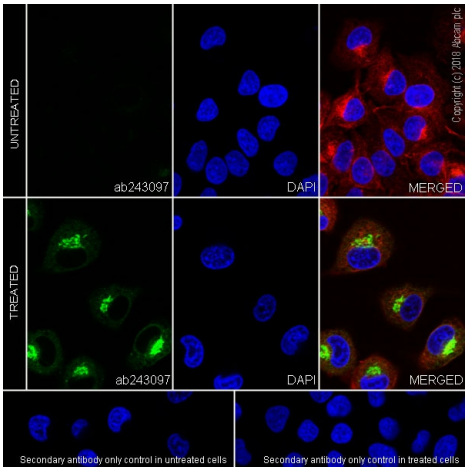
False colour image of Western blot: Anti-CXCL5 + CXCL6 antibody [EPR22310-196] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab243097 was shown to bind specifically to CXCL5 + CXCL6. A band was observed at 12 kDa in treated wild-type A549 cell lysates with no signal observed at this size in CXCL6 knockout cell line **ab275838** (knockout cell lysate **ab275812**). To generate this image, wild-type and CXCL6 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % milk in TBS-0.1 % Tween® 20 (TBS-T) 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 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Flow Cytometry (Intracellular) - Anti-CXCL5 + CXCL6 antibody [EPR22310-196] (ab243097)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized A549 (human lung carcinoma cell line) cell line that was serum starved for 4h, then treated with TNF alpha (10ng/ml 24h), PMA (10nM 24h) and BSA (0.1% 24h) (Red) / Untreated control (Green) labeling CXCL5 + CXCL6with ab243097 at 1/500 dilution, compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (Black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488)at 1/2000 dilution was used as the secondary antibody.

The expression of CXCL5 and CXCL6 is induced by TNF-a and PMA. (PMID: 9057843; 23922745).

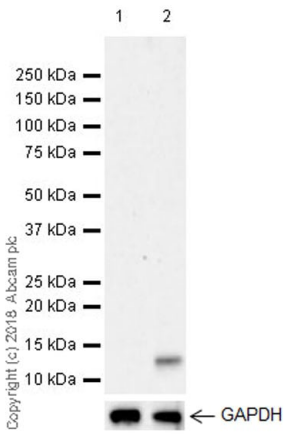


Immunocytochemistry/ Immunofluorescence - Anti-CXCL5 + CXCL6 antibody [EPR22310-196] (ab243097)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A549 (human lung carcinoma cell line) cells labeling CXCL5 + CXCL6 with ab243097 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in A549 cells treated with TNF alpha (10ng/ml 24h), PMA (10nM 24h) and BSA (0.1% 24h) is observed. Tubulin was stained using the Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594, **ab195889**) at 1/200 dilution (Red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is AlexaFluor®488 Goat anti-Rabbit secondary (**ab150077**) at 1/1000 dilution.

The expression of CXCL5 and CXCL6 is induced by TNF-a and PMA treatment. (PMID: 9057843; 23922745).



Western blot - Anti-CXCL5 + CXCL6 antibody [EPR22310-196] (ab243097)

All lanes : Anti-CXCL5 + CXCL6 antibody [EPR22310-196] (ab243097) at 1/500 dilution

Lane 1 : A549 (human lung carcinoma cell line) starved overnight, whole cell lysate
Lane 2 : A549 was starved overnight, then treated with 10ng/ml TNF-a, 10nM Phorbol-12-myristate-13-acetate (PMA) and 0.1% BSA for 24 hours, whole cell lysate

Lysates/proteins at 10 µg per lane.

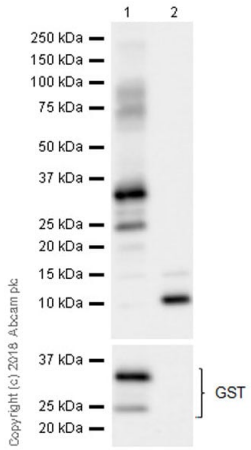
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 12 kDa

Exposure time: 3 minutes

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

The expression of CXCL5 and CXCL6 is induced by TNF-a and PMA. (PMID 9057843; PMID: 23922745).



Western blot - Anti-CXCL5 + CXCL6 antibody [EPR22310-196] (ab243097)

All lanes : Anti-CXCL5 + CXCL6 antibody [EPR22310-196] (ab243097) at 1/1000 dilution

Lane 1 : His-GST-tagged human CXCL5 recombinant protein (aa37-114) 10 ng

Lane 2 : Human CXCL6 recombinant protein (aa40-114) without tag 10 ng

Secondary

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

Predicted band size: 12 kDa

Exposure time: 3 seconds

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

Anti-CXCL5 + CXCL6 antibody [EPR22310-196] (ab243097)

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