abcam

Product datasheet

Anti-CCL4/MIP-1 beta antibody [EP521Y] ab45690

KO VALIDATED Recombinant RabMAb

10 References 8 Images

Overview

Product name	Anti-CCL4/MIP-1 beta antibody [EP521Y]
Description	Rabbit monoclonal [EP521Y] to CCL4/MIP-1 beta
Host species	Rabbit
Tested applications	**Suitable for:** ELISA, IP, Flow Cyt (Intra), WB, ICC/IF
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide within Human CCL4/MIP-1 beta aa 1 to the C-terminus (N terminal). The exact sequence is proprietary. Database link: **P13236**
Positive control	THP-1, Raw264.7
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. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP521Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab45690 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
ELISA		Use at an assay dependent concentration.
IP		1/60.
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		1/1000 - 1/500000. Detects a band of approximately 10 kDa (predicted molecular weight: 10 kDa).
ICC/IF		1/100.

Target

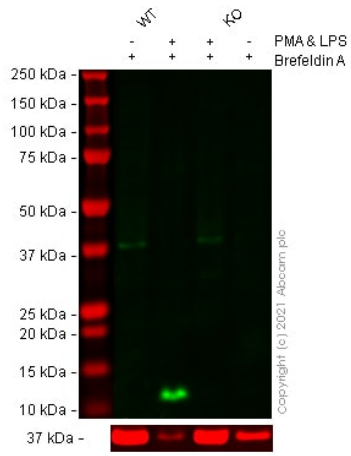
Function Monokine with inflammatory and chemokinetic properties. Binds to CCR5. One of the major HIV-suppressive factors produced by CD8+ T-cells. Recombinant MIP-1-beta induces a dose-dependent inhibition of different strains of HIV-1, HIV-2, and simian immunodeficiency virus (SIV). The processed form MIP-1-beta(3-69) retains the abilities to induce down-modulation of surface expression of the chemokine receptor CCR5 and to inhibit the CCR5-mediated entry of HIV-1 in T-cells. MIP-1-beta(3-69) is also a ligand for CCR1 and CCR2 isoform B.

Sequence similarities Belongs to the intercrine beta (chemokine CC) family.

Post-translational modifications N-terminal processed form MIP-1-beta(3-69) is produced by proteolytic cleavage after secretion from peripheral blood lymphocytes.

Cellular localization Secreted.

Images



Western blot - Anti-CCL4/MIP-1 beta antibody [EP521Y] (ab45690)

All lanes : Anti-CCL4/MIP-1 beta antibody [EP521Y] (ab45690) at 1/1000 dilution

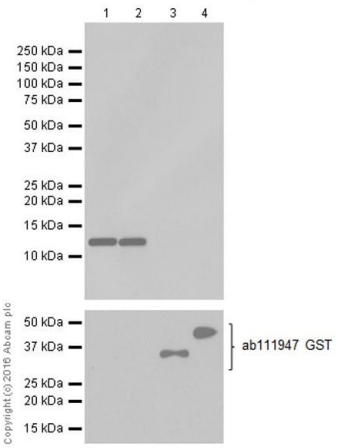
Lane 1 : Wild-type THP-1 Vehicle control + Brefeldin A (5 u/mL, 6 h) cell lysate
Lane 2 : Wild-type THP-1 Treated PMA (100 ng/mL, 56 h) + LPS (1 u/mL, 24 h) + Brefeldin A (5 u/mL, 6 h) cell lysate
Lane 3 : CCL4 knockout THP-1 Vehicle control + Brefeldin A (5 u/mL, 6 h) cell lysate
Lane 4 : CCL4 knockout THP-1 Treated PMA (100 ng/mL, 56 h) + LPS (1 u/mL, 24 h) + Brefeldin A (5 u/mL, 6 h) cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 10 kDa
Observed band size: 12 kDa

False colour image of Western blot: Anti-CCL4/MIP-1 beta antibody [EP521Y] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab45690 was shown to bind specifically to CCL4/MIP-1 beta. A band was observed at 12 kDa in wild-type THP-1 cell lysates with no signal observed at this size in CCL4 knockout cell line **ab273719** (knockout cell lysate **ab275512**). To generate this image, wild-type and CCL4 knockout THP-1 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % 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 (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-CCL4/MIP-1 beta antibody [EP521Y] (ab45690)

All lanes : Anti-CCL4/MIP-1 beta antibody [EP521Y] (ab45690) at 1/1000 dilution

Lane 1 : Untagged human CCL4 recombinant protein (aa24-92)
Lane 2 : Untagged human CCL4L recombinant protein (aa24-92)
Lane 3 : GST-tagged human CCL3 recombinant protein (aa27-92)
Lane 4 : GST-tagged human CCL3L recombinant protein 2*(aa28-93)

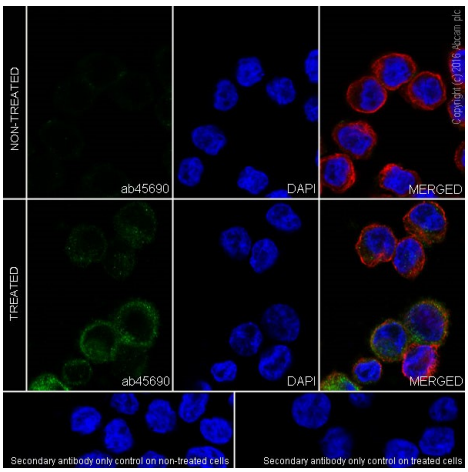
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 10 kDa
Observed band size: 12 kDa

Exposure time: 5 seconds

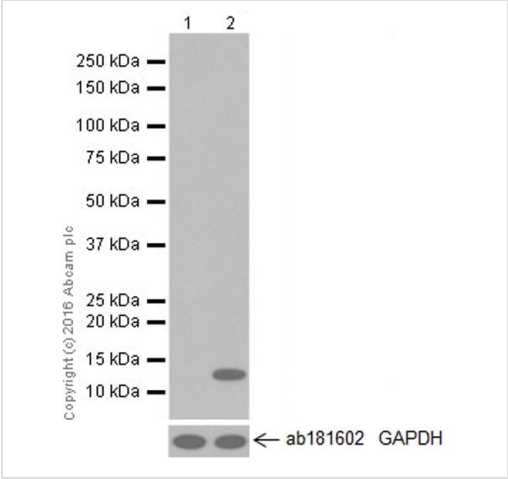
Blocking/Diluting buffer and concentration 5% NFDM /TBST



Immunocytochemistry/ Immunofluorescence - Anti-CCL4/MIP-1 beta antibody [EP521Y] (ab45690)

Immunocytochemistry/Immunofluorescence analysis of THP-1 (Human monocytic leukemia cell line) cells labeling CCL4/MIP-1 beta + CCL4L with ab45690 at 1/100. Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. Cells were counter-stained with **ab7291**, **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 dilution. DAPI was used to stain nuclei blue.

The expression increased after treatment with Lipopolysaccharides (LPS), 100 ng/mL for 4 hours, followed by addition of Brefeldin A (1 µg/mL) for 3 hours.



Western blot - Anti-CCL4/MIP-1 beta antibody [EP521Y] (ab45690)

All lanes : Anti-CCL4/MIP-1 beta antibody [EP521Y] (ab45690) at 1/1000 dilution

Lane 1 : Untreated THP-1 (human acute monocytic leukemia) cell lysate
Lane 2 : THP-1 treated with 100 nM Phorbol-12-myristate-13-acetate(PMA) overnight, then treated with Lipopolysaccharides (LPS) 100 ng/mL for 7 hours and then 1 µg/mL Brefeldin A was added for the last 3 hours, lysate

Lysates/proteins at 10 µg per lane.

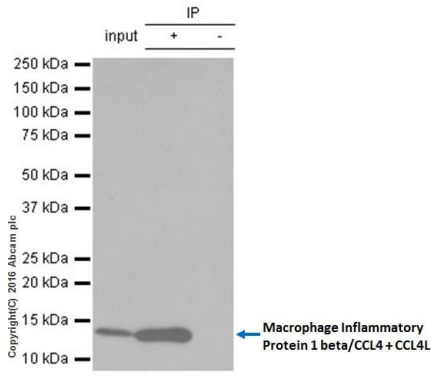
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**)

Predicted band size: 10 kDa
Observed band size: 12 kDa

Exposure time: 3 minutes

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

CCL4/MIP-1 beta is induced in macrophages following exposure to bacterial LPS (PMID: 9848081).



Immunoprecipitation - Anti-CCL4/MIP-1 beta antibody [EP521Y] (ab45690)

ab45690 at 1/60 immunoprecipitating CCL4/MIP-1 beta + CCL4L in THP-1 (Human monocytic leukemia cell line) whole cell lysate observed at 12 KDa (lanes 1 and 2).

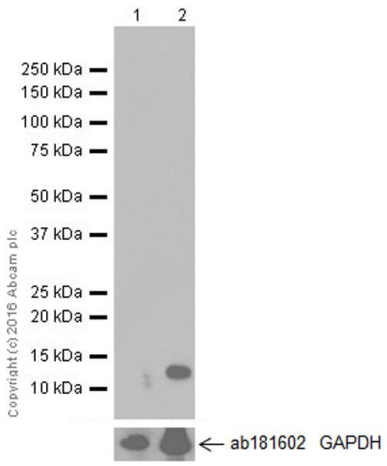
Lane 1 (input): THP-1 treated with 100 nM PMA overnight, then treated with 100 ng/mL LPS for 7 hours and 1 µg/mL Brefeldin A was added for the last 3 hours whole cell lysate, 10µg

Lane 2 (+): ab45690 + THP-1 treated with 100 nM PMA overnight, then treated with 100 ng/mL LPS for 7 hours and 1 µg/mL Brefeldin A was added for the last 3 hours whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab45690 in THP-1 treated with 100 nM PMA overnight, then treated with 100 ng/mL LPS for 7 hours and 1 µg/mL Brefeldin A was added for the last 3 hours whole cell lysate

For western blotting, ab45690 at 1/1000 and **ab131366** VeriBlot for IP Detection Reagent (HRP) was used for detection (1/1000).

Blocking/Diluting buffer 5% NFDM/TBST



Western blot - Anti-CCL4/MIP-1 beta antibody [EP521Y] (ab45690)

All lanes : Anti-CCL4/MIP-1 beta antibody [EP521Y] (ab45690) at 1/1000 dilution

Lane 1 : Untreated Raw264.7 (mouse abelson murine leukemia virus-induced tumor) whole cell lysate
Lane 2 : Raw264.7 (mouse abelson murine leukemia virus-induced tumor) treated with LPS 10µg/mL for 4 hours and then 1 µg/mL Brefeldin A was added for the last 3 hours lysate

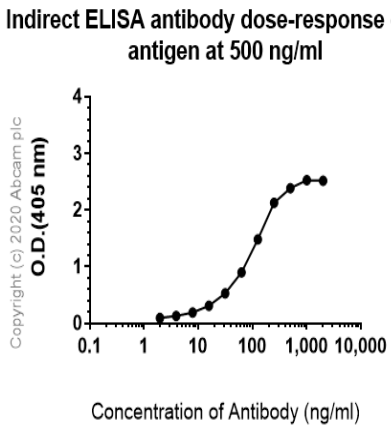
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 10 kDa

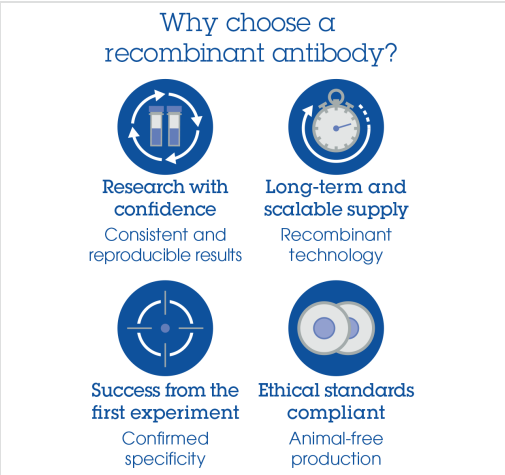
Exposure time: 3 minutes

Blocking/Diluting buffer and concentration 5% NFDM /TBST



ELISA - Anti-CCL4/MIP-1 beta antibody [EP521Y] (ab45690)

ELISA analysis of Human CCL4/MIP-1 beta recombinant protein at 500 ng/mL with ab45690. An Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) at 1/2500 dilution was used as the secondary antibody.



Anti-CCL4/MIP-1 beta antibody [EP521Y] (ab45690)

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