abcam

Product datasheet

Anti-CCL4/MIP-1 beta antibody [EP521Y] - BSA and Azide free ab187674

KO VALIDATED Recombinant RabMAb

8 Images

Overview

Product name	Anti-CCL4/MIP-1 beta antibody [EP521Y] - BSA and Azide free
Description	Rabbit monoclonal [EP521Y] to CCL4/MIP-1 beta - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** ELISA, IP, WB, ICC/IF
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
General notes	ab187674 is the carrier-free version of **ab45690**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP521Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab187674 in the following tested applications.

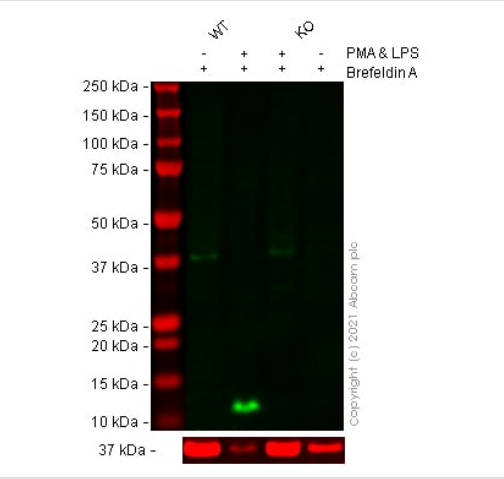
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ELISA		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 10 kDa (predicted molecular weight: 10 kDa).
ICC/IF		Use at an assay dependent concentration.

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] - BSA and Azide free (ab187674)

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

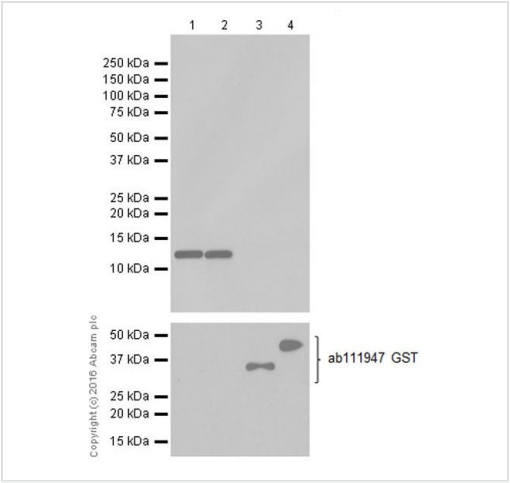
Lane 1 : Wild-type THP-1 Vehicle control + Brefeldin A (5 u/mL, 6 h) cell lysate
Lane 2 : Wild-type THP-1 Treated PMA (100 ng/mL, 56 h) + LPS (1 u/mL, 24 h) + Brefeldin A (5 u/mL, 6 h) cell lysate
Lane 3 : CCL4 knockout THP-1 Vehicle control + Brefeldin A (5 u/mL, 6 h) cell lysate
Lane 4 : CCL4 knockout 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] - BSA and Azide free (ab187674)

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.

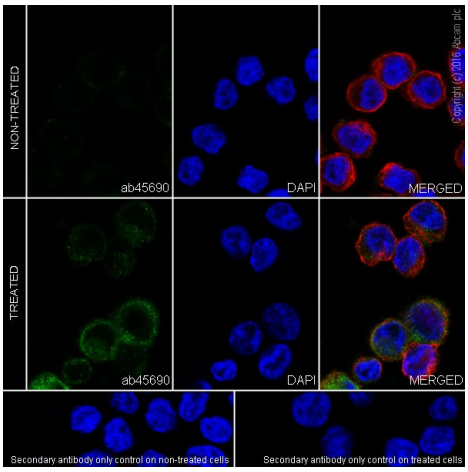
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Exposure time: 5 seconds

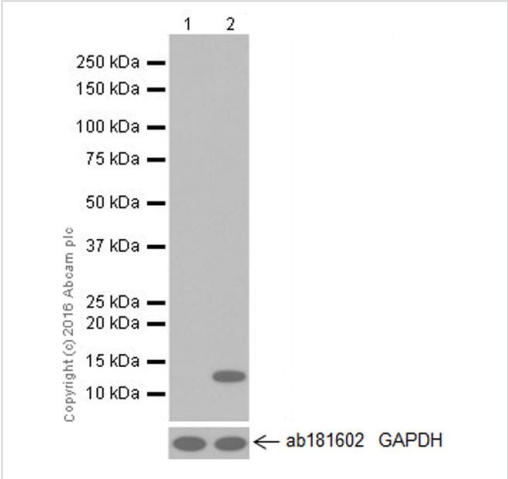
This data was developed using **ab45690**, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-CCL4/MIP-1 beta antibody [EP521Y] - BSA and Azide free (ab187674)

This data was developed using **ab45690**, the same antibody clone in a different buffer formulation.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] - BSA and Azide free (ab187674)

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

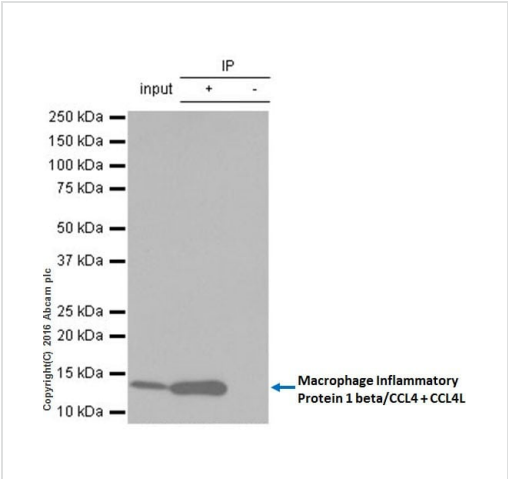
Observed band size: 12 kDa

Exposure time: 3 minutes

This data was developed using **ab45690**, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 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] - BSA and Azide free (ab187674)

This data was developed using **ab45690**, the same antibody clone in a different buffer formulation.

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.

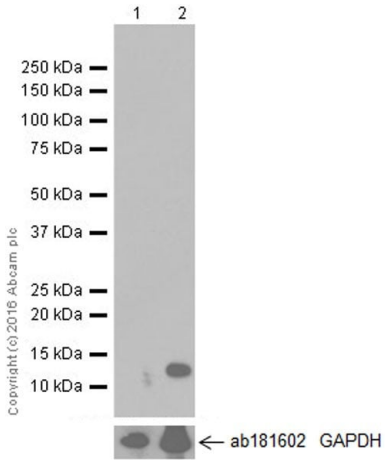
Lane 2 (+): **ab45690** + THP-1 treated with 100 nM PMA overnight, then treated with 100 ng/mL LPS for 7 hours and 1 µg/mL Brefeldin A was added for the last 3 hours whole cell lysate.

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 and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-CCL4/MIP-1 beta antibody [EP521Y] - BSA and Azide free (ab187674)

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.

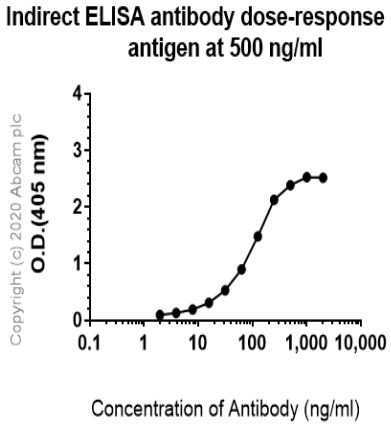
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 10 kDa

Exposure time: 3 minutes

This data was developed using **ab45690**, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.



ELISA - Anti-CCL4/MIP-1 beta antibody [EP521Y] - BSA and Azide free (ab187674)

This data was developed using **ab45690**, the same antibody clone in a different buffer formulation.

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] - BSA and Azide free (ab187674)

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