## Product datasheet

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

### Overview

<table>
<thead>
<tr>
<th><strong>Product name</strong></th>
<th>Anti-CCL4/MIP-1 beta antibody [EP521Y]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
<td>Rabbit monoclonal [EP521Y] to CCL4/MIP-1 beta</td>
</tr>
<tr>
<td><strong>Host species</strong></td>
<td>Rabbit</td>
</tr>
<tr>
<td><strong>Tested applications</strong></td>
<td>Suitable for: ELISA, IP, WB, ICC/IF</td>
</tr>
<tr>
<td><strong>Species reactivity</strong></td>
<td>Reacts with: Mouse, Human</td>
</tr>
<tr>
<td><strong>Immunogen</strong></td>
<td>Synthetic peptide within Human CCL4/MIP-1 beta aa 1 to the C-terminus (N terminal). The exact sequence is proprietary. Database link: P13236</td>
</tr>
<tr>
<td><strong>Positive control</strong></td>
<td>THP-1, Raw264.7</td>
</tr>
<tr>
<td><strong>General notes</strong></td>
<td>This product was previously labelled as Macrophage Inflammatory Protein 1 beta, MIP1 beta</td>
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</table>

This product was 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

<table>
<thead>
<tr>
<th><strong>Form</strong></th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Storage instructions</strong></td>
<td>Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.</td>
</tr>
</tbody>
</table>
| **Storage buffer** | pH: 7.20  
Preservative: 0.01% Sodium azide  
Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA |
| **Purity** | IgG fraction |
| **Clonality** | Monoclonal |
Clone number: EP521Y
Isotype: IgG

Applications

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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td>1/60.</td>
</tr>
<tr>
<td>WB</td>
<td></td>
<td>1/1000 - 1/500000. Detects a band of approximately 10 kDa (predicted molecular weight: 10 kDa).</td>
</tr>
<tr>
<td>ICC/IF</td>
<td></td>
<td>1/100.</td>
</tr>
</tbody>
</table>

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
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

why is the actual band size different from the predicted?

Exposure time: 5 seconds

Blocking/Diluting buffer and concentration 5% NFDM/TBST

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 counterstained 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) 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 **why is the actual band size different from the predicted?**

**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 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.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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