

## DESCRIPTION

<b>Species Reactivity</b>	Mouse
<b>Specificity</b>	Detects CCL4/MIP-1 $\beta$ in direct ELISAs and Western blots. In direct ELISAs, less than 5% cross-reactivity with recombinant human (rh) MIP-1 $\beta$ and recombinant mouse (rm) MIP-1 $\alpha$ is observed. This antibody will not neutralize the biological activity of rmMIP-1 $\alpha$ . In the chemotaxis assay this antibody will partially neutralize rhMIP-1 $\alpha$ and rhMIP-1 $\beta$ at a 10-30 fold higher IgG concentration.
<b>Source</b>	Polyclonal Goat IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>	<i>E. coli</i> -derived recombinant mouse CCL4/MIP-1 $\beta$ Ala24-Asn92 Accession # Q5QNV9
<b>Endotoxin Level</b>	<0.10 EU per 1 $\mu$ g of the antibody by the LAL method.
<b>Formulation</b>	Lyophilized from a 0.2 $\mu$ m filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 $\mu$ m filtered solution in PBS.

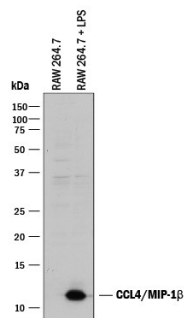
## APPLICATIONS

**Please Note:** Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
<b>Western Blot</b>	1 $\mu$ g/mL	See Below
<b>Immunohistochemistry</b>	1-15 $\mu$ g/mL	See Below
<b>Neutralization</b>	Measured by its ability to neutralize CCL4/MIP-1 $\beta$ -induced chemotaxis in the BaF3 mouse pro-B cell line transfected with human CCR5. The Neutralization Dose (ND <sub>50</sub> ) is typically 0.2-1.0 $\mu$ g/mL in the presence of 25 ng/mL Recombinant Mouse CCL4/MIP-1 $\beta$ .	

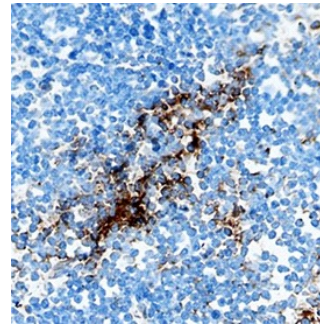
## DATA

### Western Blot



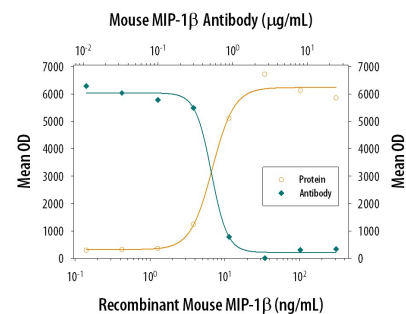
**Detection of Mouse CCL4/MIP-1 $\beta$  by Western Blot.** Western blot shows lysates of RAW 264.7 mouse monocyte/macrophage cell line untreated (-) or treated (+) with 10  $\mu$ g/mL LPS for 4 hours. PVDF membrane was probed with 1  $\mu$ g/mL of Goat Anti-Mouse CCL4/MIP-1 $\beta$  Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-451-NA) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for CCL4/MIP-1 $\beta$  at approximately 12 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

### Immunohistochemistry



**CCL4/MIP-1 $\beta$  in Mouse Small Intestine.** CCL4/MIP-1 $\beta$  was detected in perfusion fixed frozen sections of mouse small intestine using Goat Anti-Mouse CCL4/MIP-1 $\beta$  Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-451-NA) at 1.7  $\mu$ g/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). Specific staining was localized to Peyer's patches. View our protocol for [Chromogenic IHC Staining of Frozen Tissue Sections](#).

### Neutralization



**Chemotaxis Induced by CCL4/MIP-1 $\beta$  and Neutralization by Mouse CCL4/MIP-1 $\beta$  Antibody.** Recombinant Mouse CCL4/MIP-1 $\beta$  (Catalog # 451-MB) chemoattracts the BaF3 mouse pro-B cell line transfected with human CCR5 in a dose-dependent manner (orange line). The amount of cells that migrated through to the lower chemotaxis chamber was measured by Resazurin (Catalog # AR002). Chemotaxis elicited by Recombinant Mouse CCL4/MIP-1 $\beta$  (25 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Mouse CCL4/MIP-1 $\beta$  Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-451-NA). The ND<sub>50</sub> is typically 0.2-1.0  $\mu$ g/mL.

**PREPARATION AND STORAGE**

<b>Reconstitution</b>	Reconstitute at 0.2 mg/mL in sterile PBS.
<b>Shipping</b>	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>● 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>● 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>● 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

**BACKGROUND**

CCL4, also known as macrophage inflammatory protein 1 beta (MIP-1 $\beta$ ), is a 12 kDa  $\beta$  chemokine that is secreted at sites of inflammation by activated leukocytes, lymphocytes, vascular endothelial cells, and pulmonary smooth muscle cells (1, 2). CCL4 attracts a variety of immune cells to sites of microbial infection as well as to other pathologic inflammation such as allergic asthma and ischemic myocardium (3-8). A CCL4 deficiency in mice promotes the development of autoantibodies, possibly as a result of compromised regulatory T cell recruitment (6). CCL4 is secreted from activated monocytes as a heterodimer with CCL3/MIP-1 $\alpha$  (9). The first two N-terminal amino acids can be cleaved from human CCL4 by CD26/DPPIV (10, 11). Both the full length and truncated forms exert biological activity through CCR5, and the truncated form additionally interacts with CCR1 and CCR2 (10). In humans, the ability of CCL4 to bind CCR5 inhibits the cellular entry of M-tropic HIV-1 which utilizes CCR5 as a coreceptor (2). Both forms of CCL4 block HIV-1 infection of T cells by inducing the downregulation of CCR5 (10). Mature mouse CCL4 shares 77% and 86% aa sequence identity with human and rat CCL4, respectively.

**References:**

1. Rot, A. and U.H. von Andrian (2004) *Annu. Rev. Immunol.* **22**:891.
2. Menten, P. *et al.* (2002) *Cytokine Growth Factor Rev.* **13**:455.
3. Sun, X. *et al.* (2006) *Infect. Immun.* **74**:5943.
4. Bisset, L.R. and Schmid-Grendelmeier, P. (2005) *Curr. Opin. Pulm. Med.* **11**:35.
5. Frangogiannis, N.G. (2004) *Inflamm. Res.* **53**:585.
6. Bystry, R.S. *et al.* (2001) *Nat. Immunol.* **2**:1126.
7. Oliveira, S.H.P. *et al.* (2002) *J. Leukoc. Biol.* **71**:1019.
8. Schall, T.J. *et al.* (1993) *J. Exp. Med.* **177**:1821.
9. Guan, E. *et al.* (2001) *J. Biol. Chem.* **276**:12404.
10. Guan, E. *et al.* (2002) *J. Biol. Chem.* **277**:32348.
11. Guan, E. *et al.* (2004) *J. Cell. Biochem.* **92**:53.