

DESCRIPTION	
<b>Species Reactivity</b>	Canine
<b>Specificity</b>	Detects canine CCL2/JE/MCP-1 in direct ELISAs and Western blots.
<b>Source</b>	Monoclonal Mouse IgG <sub>1</sub> Clone # 280702
<b>Purification</b>	Protein A or G purified from hybridoma culture supernatant
<b>Immunogen</b>	<i>E. coli</i> -derived recombinant canine CCL2/JE/MCP-1 Gln24-Pro101 Accession # P52203
<b>Endotoxin Level</b>	<0.10 EU per 1 µg of the antibody by the LAL method.
<b>Formulation</b>	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm 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 µg/mL	Recombinant Canine CCL2/JE/MCP-1 (Catalog # 1774-MC)
<b>Immunocytochemistry</b>	8-25 µg/mL	See Below
<b>Canine CCL2/JE/MCP-1 Sandwich Immunoassay</b>		<b>Reagent</b>
<b>ELISA Capture</b>	2-8 µg/mL	Canine CCL2/JE/MCP-1 Antibody (Catalog # MAB28171)
<b>ELISA Detection Standard</b>	0.1-0.4 µg/mL	Canine CCL2/JE/MCP-1 Biotinylated Antibody (Catalog # BAF1774) Recombinant Canine CCL2/JE/MCP-1 (Catalog # 1774-MC)
<b>Neutralization</b>	Measured by its ability to neutralize CCL2/JE/MCP-1-induced chemotaxis in the BaF3 mouse pro-B cell line transfected with human CCR2A. The Neutralization Dose (ND <sub>50</sub> ) is typically 0.5-2.0 µg/mL in the presence of 300 ng/mL Recombinant Canine CCL2/JE/MCP-1.	

## DATA

**Neutralization**

**Chemotaxis Induced by CCL2/JE/MCP-1 and Neutralization by Canine CCL2/JE/MCP-1 Antibody.** Recombinant Canine CCL2/JE/MCP-1 (Catalog # 1774-MC) chemoattracts the BaF3 mouse pro-B cell line transfected with human CCR2A 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 Canine CCL2/JE/MCP-1 (300 ng/mL) is neutralized (green line) by increasing concentrations of Mouse Anti-Canine CCL2/JE/MCP-1 Monoclonal Antibody (Catalog # MAB28171). The ND<sub>50</sub> is typically 0.5-2.0 µg/mL.

**Immunocytochemistry**

**CCL2/JE/MCP-1 in Canine PBMCs.** CCL2/JE/MCP-1 was detected in immersion fixed canine peripheral blood mononuclear cells (PBMCs) using Mouse Anti-Canine CCL2/JE/MCP-1 Monoclonal Antibody (Catalog # MAB28171) at 25 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for [Fluorescent ICC Staining of Non-adherent Cells](#).

## PREPARATION AND STORAGE

<b>Reconstitution</b>	Reconstitute at 0.5 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**

Canine MCP-1 (monocyte chemotactic protein-1) is an 8 kDa member of the CC chemokine family of chemotactic factors (1, 2). It is synthesized as a 101 amino acid (aa) precursor that contains a 23 aa signal sequence and a 78 aa mature segment (3). It contains no potential N-linked glycosylation sites and is not known for any posttranslational modifications. Based on human studies, MCP-1 will primarily circulate as a monomer. Noncovalent dimers are likely to be found, however. MCP-1 activity has been localized to the N-terminus (1). Cell types known to secrete MCP-1 are considerable in number, and include keratinocytes, fibroblasts, endothelium, osteoblasts, macrophages, mast cells, smooth muscle cells, and astrocytes (1, 2). In the mature MCP-1 segment, there is 82% and 83% aa identity, canine to human and porcine MCP-1, respectively. When mature canine MCP-1 is compared to (125 aa) extended rodent MCP-1, there is 55% and 56% aa identity, canine to mouse and rat MCP-1, respectively. MCP-1 has three possible receptors. The first two are CCR2 (1) and CCR11 (4). The third receptor has only been identified in mice and is called L-CCR (5). Its function is unknown. MCP-1 is best known as a chemotactic agent for mononuclear cells. It also, however, induces enzyme and cytokine release in monocytes, NK cells, and lymphocytes and histamine release by basophils (1). Additionally, it is believed to reduce IL-12 production by dendritic cells and promote a Th2 phenotype in CD4<sup>+</sup> T cells (6).

**References:**

1. Coillie, E.V. *et al.* (1999) Cytokine Growth Factor Rev. **10**:61.
2. Yoshie, O. *et al.* (2001) Adv. Immunol. **78**:57.
3. Kumar, A.G. *et al.* (1997) Circulation **95**:693.
4. Biber, K. *et al.* (2003) J. Leukoc. Biol. **74**:243.
5. Luther, S.A. and J.G. Cyster (2001) Nat. Immunol. **2**:102.