

DESCRIPTION

Species Reactivity	Rat
Specificity	Detects rat CCL4/MIP-1 β in direct ELISAs and Western blots. In direct ELISAs, approximately 8% cross-reactivity with recombinant mouse CCL4/MIP-1 β and recombinant rat CCL3/MIP-1 α is observed, and less than 2% cross-reactivity with recombinant human CCL4/MIP-1 β is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant rat CCL4/MIP-1 β Ala24-Asn92 Accession # P50230
Formulation	Lyophilized from a 0.2 μ m filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 μ 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
Western Blot	1 μ g/mL	See Below
Neutralization	Measured by its ability to neutralize CCL4/MIP-1 β -induced chemotaxis in the BaF3 mouse proB cell line transfected with human CCR5. The Neutralization Dose (ND ₅₀) is typically 0.1-0.5 μ g/mL in the presence of 0.075 μ g/mL Recombinant rat CCL4/MIP-1 β .	

DATA

Western Blot

Detection of Rat CCL4/MIP-1 β by Western Blot. Western blot shows lysates of NR8383 rat alveolar macrophage cell line untreated (-) or treated (+) with 10 μ g/mL LPS for 4 hours. PVDF membrane was probed with 1 μ g/mL of Sheep Anti-Rat CCL4/MIP-1 β Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6935) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for CCL4/MIP-1 β at approximately 12 kDa (as indicated). This experiment was conducted under reducing conditions and using [Immunoblot Buffer Group 1](#).

Neutralization

Chemotaxis Induced by CCL4/MIP-1 β and Neutralization by Rat CCL4/MIP-1 β Antibody. Recombinant rat CCL4/MIP-1 β (Catalog # 6935-MB) induces BaF3 mouse proB 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 rat CCL4/MIP-1 β (0.075 μ g/mL) is neutralized (green line) by increasing concentrations of Sheep Anti-Rat CCL4/MIP-1 β Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6935). The ND₅₀ is typically 0.1-0.5 μ g/mL.

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
Shipping	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
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

CCL4, also known as macrophage inflammatory protein 1 beta (MIP-1 β), is a 12 kDa β 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 α (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 down-regulation of CCR5 (10). Mature rat CCL4 shares 80% and 86% aa sequence identity with human and mouse CCL4, respectively.

References:

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