Anti-cotton rat CCL4/MIP-1β Antibody

ORDERING INFORMATION

Catalog Number: AF1024

Lot Number: GFB03

Size: 100 μg

Formulation: 0.2 μm filtered solution in PBS with 5% trehalose

Storage: -20° C

Reconstitution: sterile PBS

Specificity: cotton rat CCL4

Immunogen: *E. coli*-derived rcrCCL4

Ig Type: goat IgG

Applications: Neutralization of bioactivity
Western blot
Direct ELISA

Preparation
Produced in goats immunized with purified, *E. coli*-derived, recombinant cotton rat CCL4 (rcrCCL4; Ala24 - Asn92; Accession # AAL16933). Cotton rat CCL4 specific IgG was purified by cotton rat CCL4 affinity chromatography.

Formulation
Lyophilized from a 0.2 μm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Endotoxin Level
< 0.01 EU per 1 μg of the antibody as determined by the LAL method.

Reconstitution
Reconstitute with sterile PBS. If 1 mL of PBS is used, the antibody concentration will be 0.1 mg/mL.

Storage
Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C **in a manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity
This antibody was selected for its ability to neutralize cotton rat CCL4 bioactivity.

Neutralization of Cotton Rat CCL4 bioactivity
The exact concentration of antibody required to neutralize rcrCCL4 activity is dependent on the cytokine concentration, cell type, growth conditions and the type of activity studied. To provide a guideline, R&D Systems has determined the neutralization dose for this antibody under a specific set of conditions. The **Neutralization Dose$_{50}$ (ND$_{50}$)** for this antibody is defined as that concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when that cytokine is present at a concentration just high enough to elicit a maximum response.

The ND$_{50}$ for this lot of anti-cotton rat CCL4 antibody was determined to be approximately 0.15 - 0.6 μg/mL in the presence of 10 ng/mL of rcrCCL4, using the mCCR5 transfected BaF/3 cell chemotaxis assay. The specific conditions are described in the figure legends.

Additional Applications
Western blot - This antibody can be used at 1 - 2 μg/mL with the appropriate secondary reagents to detect cotton rat CCL4. The detection limit for rcrCCL4 is approximately 2 ng/lane under non-reducing and reducing conditions.

Direct ELISA - This antibody can be used at 0.5 - 1.0 μg/mL with the appropriate secondary reagents to detect cotton rat CCL4. The detection limit for rcrCCL4 is approximately 0.5 ng/well. In this format, this antibody shows approximately 25% cross-reactivity with rhMIP-1β and less than 2% cross-reactivity with rmMIP-1β.

Optimal dilutions should be determined by each laboratory for each application.

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1-800-343-7475

Figure 1

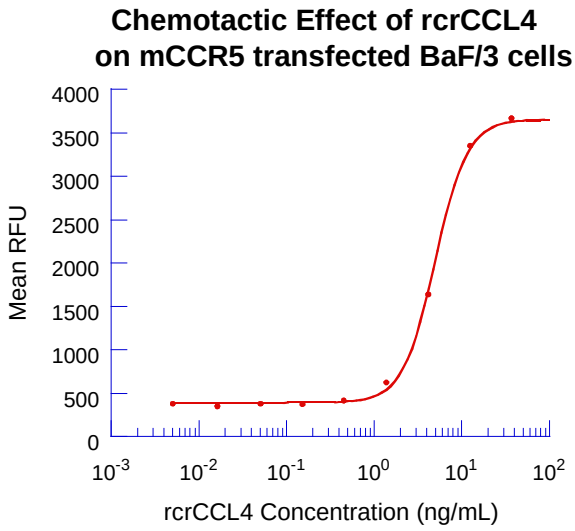

Figure 2

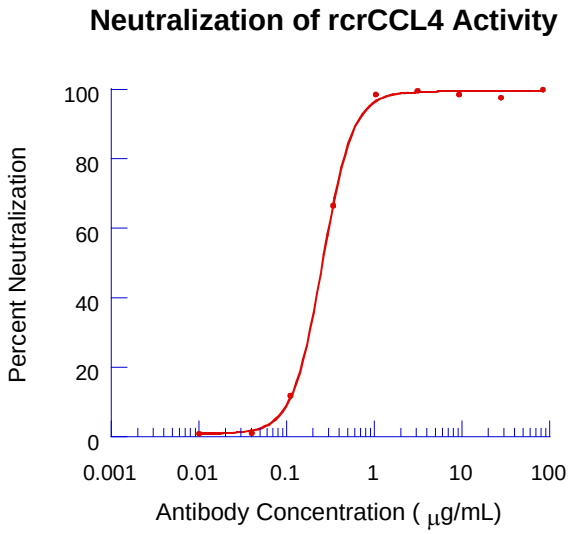

Figure 1
Cotton rat CCL4 chemoattracts mCCR-5 transfected BaF/3 cells. The ED_{50} for this effect is typically 1 - 5 ng/mL.

Figure 2
To measure the ability of the antibody to neutralize the chemoattractant activity of rcrCCL4 for BaF/3 mCCR-5 cells, rcrCCL4 was incubated with various concentrations of the antibody for 30 minutes at room temperature in a 96-well microplate. Following this preincubation period, 75 μL of the cytokine-antibody solution (containing rcrCCL4 at a final concentration of 10 ng/mL and antibody at the concentrations indicated) was transferred to the lower compartment of a 96-well chemotaxis chamber (NeuroProbe, Cabin John, MD). The chemotaxis chamber was then assembled using a PVP-free polycarbonate filter (5 micron pore size) and 0.25 x 10^6 cells/well was added to the top chamber. After incubation for 3 hours at 37° C in a 5% CO_2 humidified incubator, the chamber was disassembled and the cells that migrated through to the lower chamber were transferred to a working plate and stained using Resazurin (R&D Systems, Catalog # AR002). The relative fluorescence was read with excitation wavelenth set at 544 nm and emission at 590 nm. As shown in Figure 2, the ND_{50} for this lot of antibody is approximately 0.15 - 0.6 μg/mL.