

ORDERING INFORMATION

Catalog Number: AF364

Lot Number: ARY01

Size: 100 µg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human CCL17

Immunogen: *E. coli*-derived rhCCL17

Ig Type: goat IgG

Applications: Neutralization of bioactivity
Direct ELISA
Western blot
Immunohistochemistry

Preparation

Produced in goats immunized with purified, *E. coli*-derived, recombinant human CCL17 (rhCCL17). CCL17 specific IgG was purified by human CCL17 affinity chromatography.

Formulation

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

Endotoxin Level

< 0.1 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 has been selected for its ability to neutralize the biological activity of rhCCL17. Based on direct ELISA results, this antibody shows no cross-reactivity with other chemokines tested.¹

Neutralization of Human CCL17 Bioactivity

The exact concentration of antibody required to neutralize rhCCL17 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 Dose₅₀ (ND₅₀)** 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 ND₅₀ for this lot of anti-human CCL17 antibody was determined to be approximately 0.3 - 1.5 µg/mL in the presence of 0.01 µg/mL of rhCCL17, using human CCR4 transfected BaF/3 cells in a chemotaxis assay. The specific conditions are described in the figure legends.

Additional Applications

Direct ELISA - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect human CCL17. The detection limit for rhCCL17 is approximately 0.1 ng/well.

Western blot - This antibody can be used at 0.1 - 0.2 µg/mL with the appropriate secondary reagents to detect human CCL17. The detection limit for rhCCL17 is approximately 5 ng/lane under non-reducing and reducing conditions.

Immunohistochemistry - This antibody will detect CCL17 in paraffin-embedded tissue sections. The working dilution is 5 - 15 µg/mL. For chromogenic detection of labeling, use R&D Systems' Cell and Tissue Staining Kits (CTS Series).

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

For immunohistochemistry images, please refer to our website at <http://www.rndsystems.com/ihc>

Figure 1

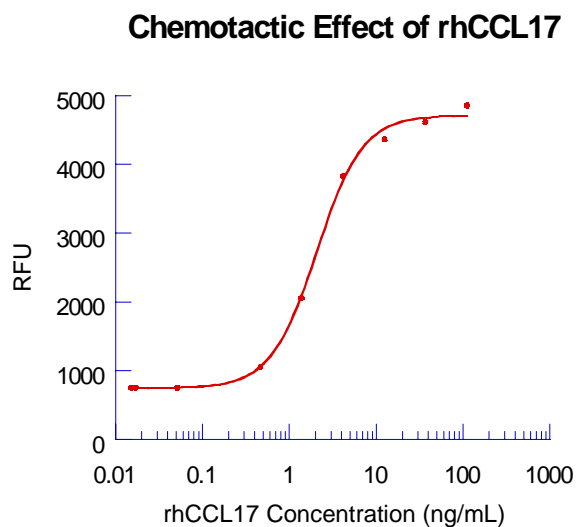


Figure 2

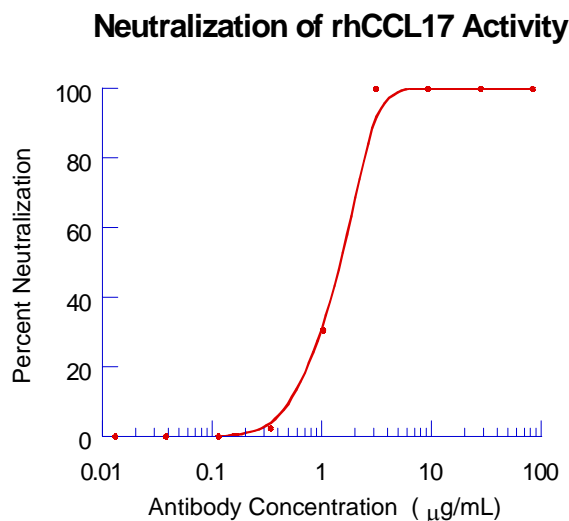


Figure 1

Human CCL17 chemoattracts hCCR4 transfected BaF/3 cells. The number of cells that have migrated through to the lower chamber are quantitated using Resazurin (R&D Systems, Catalog # AR002) staining. The ED₅₀ for this effect is typically 2 - 6 ng/mL.

Figure 2

To measure the ability of the antibody to neutralize the chemoattractant activity of rhCCL17 for hCCR4 transfected BaF/3 cells, rhCCL17 was incubated with various concentrations of antibody for 30 minutes at room temperature in a 96-well microplate. Following this preincubation period, 75 µL of the cytokine-antibody solution (containing rhCCL17 at a final concentration of 0.01 µg/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 0.2×10^6 cells/well was added to the top chamber. After incubation for 3 hours at 37° C in a 5% CO₂ 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 then read in a fluorescent plate reader set at Ex. 544/Em. 590. As shown in Figure 2, the ND₅₀ for this lot of antibody is approximately 0.3 - 1.5 µg/mL.

¹rh6Ckine, rm6Ckine, rmC10, rrCINC-1, rhCXC-X, rhENA-78, rhEotaxin, rmEotaxin, rhFractalkine, rhGCP-2, rmGCP-2, rhGRO α , rhGRO β , rhGRO γ , rhHCC-1, rhI-309, rhIL-8, rhIP-10, rmJE, rmKC, rmMARC, rhMCP-1, rhMCP-2, rhMCP-3, rhMIG, rmMIG, rhMIP-1 α , rmMIP-1 α , rhMIP-1 β (rhACTII), rhMIP-1 γ , rmMIP-1 γ , rhMIP-3 α , rhMIP-3 β , rhNAP-2, rhRANTES, rmRANTES, rhSDF-1 α , rhSDF-1 β , rhTeck, rmTeck, rhVIC, rmVIC