Anti-human CCL15/MIP-1δ/LKN-1 Antibody

ORDERING INFORMATION

Catalog Number: AF628

Lot Number: CNO01

Size: 100 μg

Formulation: 0.2 μm filtered solution in PBS

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human CCL15/LKN-1

Immunogen: *E. coli*-derived rhCCL15/LKN-1

Ig Type: goat IgG

Applications: Neutralization of bioactivity
Western blot
Direct ELISA

Preparation

Produced in goats immunized with the purified, 68 amino acid (aa) amino-terminally truncated isoform of *E. coli*-derived, recombinant human CCL15 (rhCCL15). CCL15 specific IgG was purified by affinity chromatography using an immobilized 68 aa amino-terminally truncated isoform CCL15/LKN-1 column.

Formulation

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

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 the 68 aa isoform of rhCCL15/LKN-1. It will also neutralize the biological activity of the 92 aa residue isoform of rhCCL15/LKN-1 using a 2-fold higher Ig concentration. In direct ELISAs and western blots, this antibody shows a preference for the 68 aa residue isoform over the 92 aa residue isoform of human CCL15/LKN-1. Additionally, in direct ELISAs, this antibody shows no cross-reactivity with other chemokines tested.[1]

Neutralization of Human CCL15/LKN-1 Bioactivity

The exact concentration of antibody required to neutralize human CCL15/LKN-1 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-human CCL15/LKN-1 antibody was determined to be approximately 0.5 - 2.5 μg/mL in the presence of 15 ng/mL of rhCCL15/LKN-1 aa 46 - 113), using the hCCR1 transfected BaF/3 cell line. 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 CCL15. The detection limit for rhCCL15 is approximately 0.3 ng/well.

Western blot - This antibody can be used at 0.1 - 0.2 μg/mL with the appropriate secondary reagents to detect human CCL15. The detection limit for rhCCL15 is approximately 0.5 ng/lane under non-reducing and reducing conditions.

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

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1-800-343-7475

Figure 1

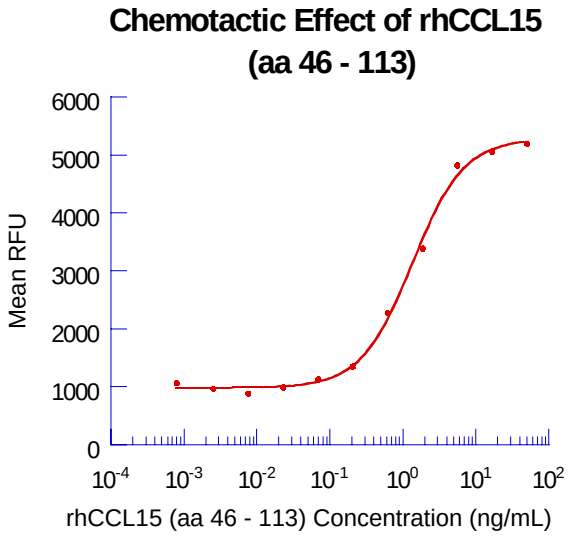

Figure 2

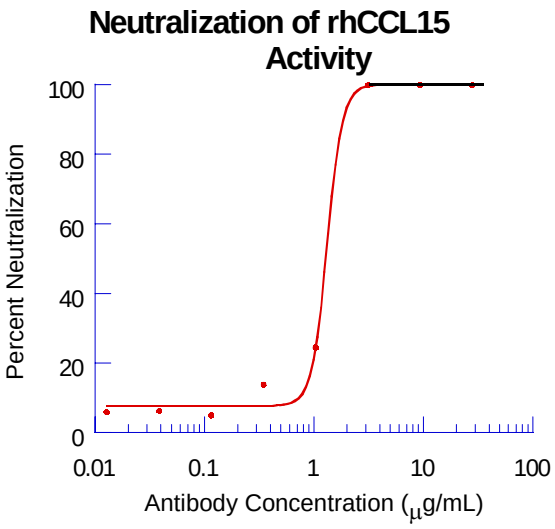

Figure 1
The 68 amino acid (aa) isoform of human CCL15/LKN-1 (R&D Systems, Catalog # 628-LK) chemoattracts hCCR1 transfected BaF/3 cells. The number of cells that have migrated through to the lower chamber are quantitated using Resazurin (R&D Systems, Catalog # AR002) staining. The ED_{50} for this effect is typically 0.6 - 3.0 ng/mL.

Figure 2
To measure the ability of the antibody to neutralize the chemoattractant activity of rhCCL15/LKN-1 for BaF/3 hCCR1 cells, rhCCL15/LKN-1 (aa 46 - 113; R&D Systems, Catalog # 628-LK) 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 rhCCL15/rhLKN-1 at a final concentration of 15 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 2.5 x 10^5 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 # AR001). The relative fluorescence was then read in a 96-well spectrofluorimeter with excitation wavelength set at 544 nm and emission at 590 nm. As shown in Figure 2, the ND_{50} for this lot of antibody is approximately 0.5 - 2.5 μg/mL.

[1]rh6Ckine, rm6Ckine, rmC10, rrCINC-1, rrCINC-2α, rrCINC-2β, rhBLC/BCA-1, rmBLC, rvCMV UL146, rhENA-78, rhEotaxin, rmEotaxin, rhEotaxin-2, rhFractalkine, rmFractalkine, rhGCP-2, rmGCP-2, rhGROα, rhGROβ, rhGROγ, rhHCC-1, rhHCC-4, rhI-309, rhIL-8, rhIP-10, rhI-TAC, rmJE, rmKC, rmLymphotactin, rmMARC, rhMCP-1, rhMCP-2, rhMCP-3, rhMCP-4, rmMCP-5, rmMDC, rhMIG, rmMIG, rhMIP-1α, rmMIP-1α, rhMIP-1β, rmMIP-1β, rvMIP-I, rmMIP-2, rhMIP-3α, rrMIP-3α, rhMIP-3β, rmMIP-3β, rvMIP-III, rhNAP-2, rhPARC, rhRANTES, rmRANTES, rhSDF-1α, rmSDF-1α, rhSDF-1β, rhTarc, rmTCA-3, rhTECK, rmTECK