Anti-viral CCI Antibody

ORDERING INFORMATION

Catalog Number: AF696

Lot Number: DGP02

Size: 100 μg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: viral CCI

Immunogen: *Sf* 21-derived rvCCI

Ig Type: goat IgG

Applications: Neutralization of bioactivity
Western blot
Direct ELISA

Preparation
Produced in goats immunized with purified, *Sf* 21-derived, recombinant viral CC Chemokine Inhibitor (rvCCI). CCI specific IgG was purified by vaccinia virus-derived CCI 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 rvCCI bioactivity.

Neutralization of Viral CCI bioactivity
The exact concentration of antibody required to neutralize viral CCI 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-viral CCI antibody was determined to be approximately 3 - 15 μg/mL in the presence of 0.4 μg/mL of rvCCI and 0.02 μg/mL of rmCCL2/JE, using the hCCR2A transfected BaF/3 cell line. The specific conditions are described in the figure legends.

Additional Applications
Western blot - This antibody can be used at 0.1 - 0.2 μg/mL with the appropriate secondary reagents to detect viral CCI. The detection limit for rvCCI is approximately 0.5 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 viral CCI. The detection limit for rvCCI is approximately 0.15 ng/well.

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

Figure 1

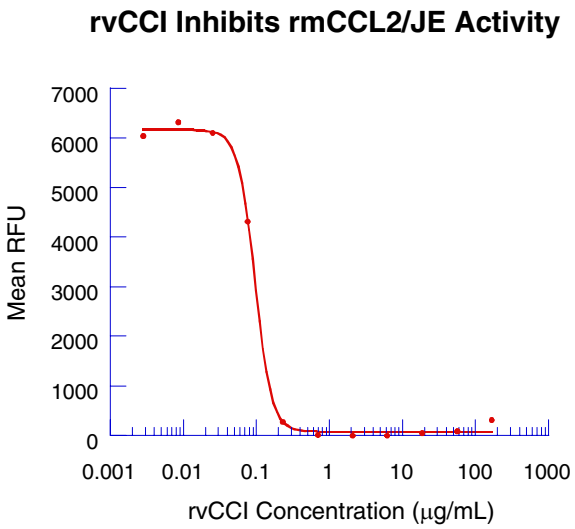

Figure 2

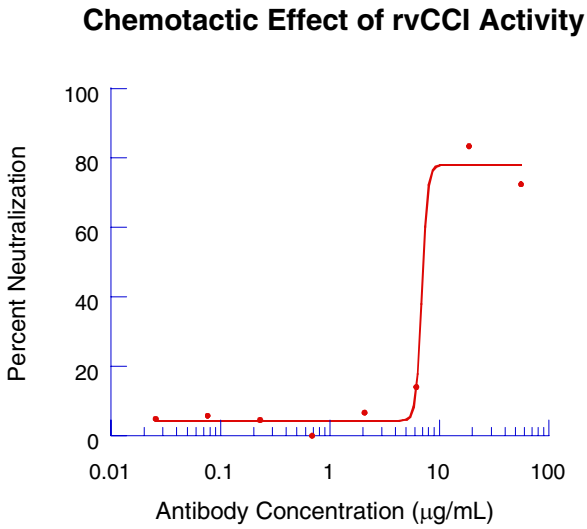

Figure 1
Viral CCI inhibits rmCCL2 (0.02 μg/mL) activity on hCCR2A transfected BaF/3 cells. The ED_{50} for this effect is typically 0.1 - 0.5 μg/mL.

Figure 2
To measure the ability of the antibody to neutralize the chemoattractant inhibition activity of rvCCI for hCCR2A transfected BaF/3 cells, rvCCI 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 rvCCI at a final concentration of 0.4 μg/mL, rmCCL2 at a final concentration of 0.02 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 4 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 # AR002). 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 3 - 15 μg/mL.