Anti-viral MIP-I Antibody

ORDERING INFORMATION

Catalog Number: AF799

Lot Number: BCQ01

Size: 100 μg

Formulation: 0.2 μm filtered solution in PBS

Storage: -20° C

Reconstitution: sterile PBS

Specificity: viral MIP-I

Immunogen: *E. coli*-derived rvMIP-I

Ig Type: goat IgG

Applications: Neutralization of bioactivity
Western blot
Direct ELISA

Preparation

Produced in goats immunized with purified, *E. coli*-derived, recombinant human viral macrophage inflammatory protein I (rvMIP-I). MIP-I specific IgG was purified by viral MIP-I affinity chromatography.

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 rvMIP-I. In direct ELISA and western blots, this antibody shows approximately 2% cross-reactivity with rvMIP-II.

Neutralization of Viral MIP-I Bioactivity

The exact concentration of antibody required to neutralize viral MIP-I 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 MIP-I antibody was determined to be approximately 0.8 - 4.0 μg/mL in the presence of 0.02 μg/mL of rvMIP-I measuring chemotaxis of hCCR8 transfected BaF/3 cells. 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 MIP-I. The detection limit for rvMIP-I is approximately 2 ng/lane under both 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 MIP-I. The detection limit for rvMIP-I is approximately 0.3 ng/well.

Optimal dilutions should be determined by each laboratory for each applicatio

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Figure 1

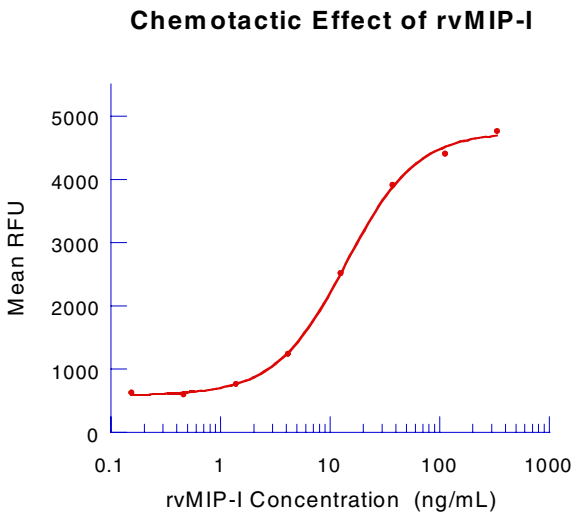

Figure 2

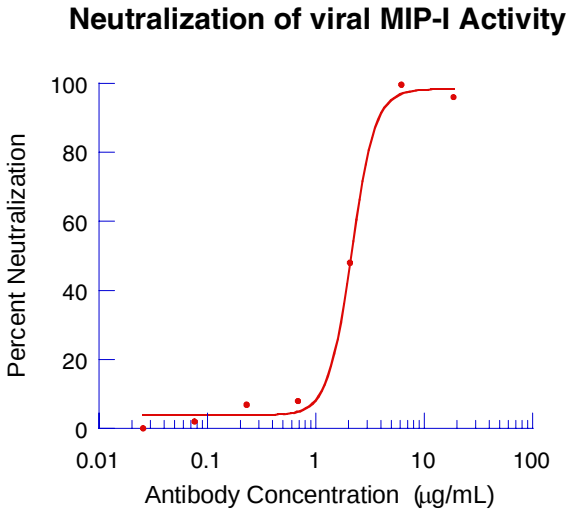

Figure 1
Viral MIP-I chemoattracts hCCR8 transfected BaF/3 cells. The ED_{50} for this effect is typically 4 - 16 ng/mL.

Figure 2
Typical data for anti-viral MIP-I is shown in Figure 2. To measure the ability of the antibody to neutralize the chemoattractant activity of rvMIP-I using hCCR8 transfected BaF/3 cells, rvMIP-I 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 rvMIP-I 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 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 carefully disassembled. The cells that migrate through to the lower chamber were transferred to a 96 well plate. Chemotaxis was measured by Resazurin (R&D Systems, Catalog # AR002) staining of cells that have migrated through the filter. The relative fluorescence is read 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 0.8 - 4.0 μg/mL.