



Monoclonal Anti-human CCR8 Antibody

ORDERING INFORMATION

Catalog Number: MAB1429

Clone: 191704

Lot Number: HUC02

Size: 500 µg

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

Storage: -20° C

Specificity: human CCR8

Reconstitution: sterile PBS

Immunogen: BaF/3 cells transfected with hCCR8

Ig class: rat IgG_{2b}

Application: Neutralization of bioactivity

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a rat immunized with BaF/3 cells transfected with human CCR8 (hCCR8). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography.

Formulation

Lyophilized from a 0.2 µm filtered solution of 5% trehalose 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 500 µg/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 the bioactivity of rhCCR8 and for its capacity to react specifically with hCCR8-transfected cell lines and not with the parent cell lines.

Application

Neutralization of Human CCR8 bioactivity

The exact concentration of antibody required to neutralize rhI-309 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 anti-human CCR8 antibody was determined to be approximately 0.01 - 0.05 µg/mL in the presence of 20 ng/mL of rhI-309, using chemoattraction of the hCCR8 transfected BaF/3 cell line as an assay. The specific conditions are described in the figure legends.

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

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

R&D Systems, Inc.
1-800-343-7475

Figure 1

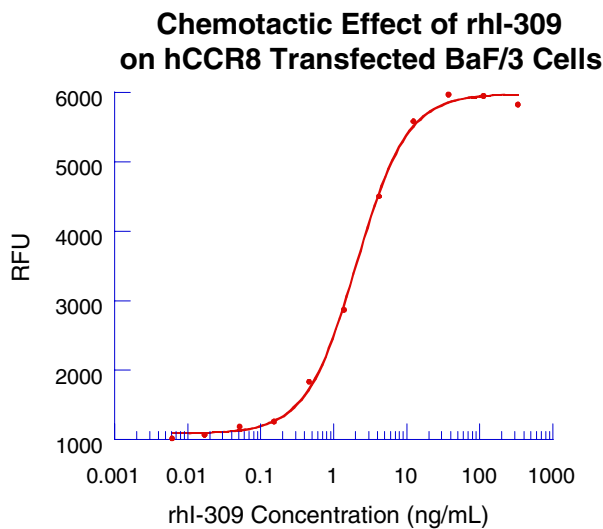


Figure 2

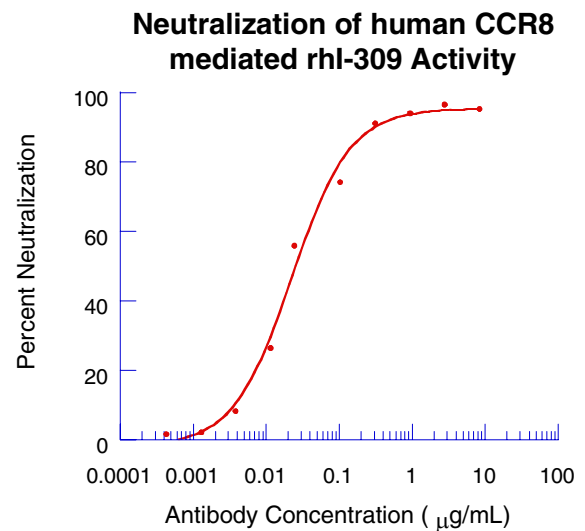


Figure 1: Human I-309 chemoattracts BaF/3 cells that have been transfected with hCCR8. The number of cells that have migrated through to the lower chamber are quantitated using Resazurin (R&D Systems, Catalog # AR002) Fluorescence staining. The ED_{50} for this effect is typically 1.5 - 7.5 ng/mL.

Figure 2: To measure the ability of the antibody to block rhI-309 mediated chemotaxis of hCCR8-transfected BaF/3 cells, rhI-309 at 20 ng/mL was added 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). Serial dilutions of the antibody (at the concentrations indicated) and 0.25×10^6 cells/well were added to the top wells of the 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 quantitated using Resazurin (R&D Systems, Catalog # AR002) Fluorescence. As shown in Figure 2, the ND_{50} for this lot of antibody is approximately 0.01 - 0.05 µg/mL.