NEUROMICS

CCR5 Data Sheet

Catalog Number:	MO15077	**Host:**	Mouse
Product Type:	Protein G purified IgG_{2A}	**Species Reactivity:**	Human
Immunogen Sequence:	Hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a Balb/c mouse inoculated with CC chemokine receptor 5 (hCCR5) transfected NS0 mouse myeloma cells.	**Format:**	Liquid 1mg/ml Solution in phosphate-buffered saline (PBS) with 5% Trehlose
Applications:	Immunohistochemistry -10 µg/mL Flow Cytometry-5-10 µg/mL		

Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.

Storage: Antibody can be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. The antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. *Avoid repeated freeze-thaw cycles.*

Application Notes

Specificity
This antibody was selected for its ability to react with hCCR5 transfectants but not the parental cell lines as detected by flow cytometry. It has also been shown to react with a CCR5/CCR-2 chimera (2252) containing the 2nd extracellular loop of hCCR5. This antibody can be used to detect CCR5 present on stimulated human PBMCs.

Immunocytochemistry
This antibody was used at a concentration of 10 µg/mL.

Neutralization of Human Cell Surface CCR5 Mediated Bioactivity
The exact concentration of antibody required to neutralize the human cell surface CCR5 mediated bioactivity is dependent on the concentration, as well as on the number of CCR5 receptors present on the cell surface (a function of cell type and culture 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 cell surface CCR5 mediated rhMIP-1α response on responsive cells at a specific rhMIP-1α concentration. The ND_{50} for this lot of anti-human CCR5 antibody was determined to be approximately 5 - 20 µg/mL in the presence of 40 ng/mL rhMIP-1α in a chemotaxis assay using BaF/3 cells transfected with hCCR5. The specific conditions are described in the figure legends.

Flow Cytometry
Dilute this antibody to 5 - 10 µg/mL and add 10 µL of this solution to 1 - 5 x 10_5 cells in a total reaction volume not exceeding 200 µL.

FOR RESEARCH USE ONLY

2/07v1

www.neuromics.com

Neuromics Antibodies • 5325 West 74th Street, Suite 8 • Edina, MN 55439
phone 866-350-1500 • fax 612-677-3976 • e-mail pshuster@neuromics1.com

Figure 1

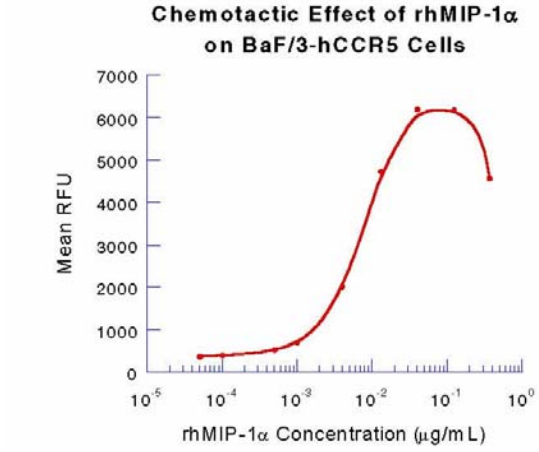

Figure 2

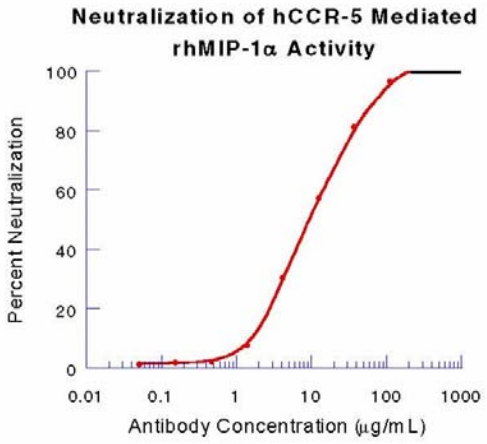

Figure 1

Human MIP-1α chemoattracts BaF/3-hCCR5 cells. The number of cells that have migrated through to the lower chamber are quantitated using Resazurin staining. The ED_{50} for this effect is typically 0.003 - 0.01 µg/mL.

Figure 2

To measure the ability of the antibody to block rhMIP-1α mediated chemotaxis of BaF/3 hCCR5 cells, rhMIP-1α at 40 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 x 10^6 cells/well were added to the top wells of the 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 quantitated using Resazurin Fluorescence. As shown in Figure 2, the ND_{50} for this lot of antibody is approximately 5 - 20 µg/mL.

FOR RESEARCH USE ONLY

2/07v1

Neuromics Antibodies • 5325 West 74th Street, Suite 8 • Edina, MN 55439
phone 866-350-1500 • fax 612-677-3976 • e-mail pshuster@neuromics1.com