

### Neutralization of Human Cell Surface CXCR2 mediated Bioactivity

The exact concentration of antibody required to neutralize human cell surface CXCR2 mediated bioactivity is dependent on the concentration as well as on the number of CXCR2 receptors present on the cell surface (a function of cell type and culture conditions). The Neutralization Dose50 (ND50) for this antibody is defined as that concentration of antibody required to yield one-half maximal inhibition of the cell surface CXCR2 mediated rhGRO $\alpha$  response on responsive cells at a specific GRO $\alpha$  concentration. The ND50 for this lot of anti-human CXCR2 antibody was determined to be approximately 1 - 5  $\mu$ g/mL in the presence of 5 ng/mL rhGRO $\alpha$  in a chemotaxis assay using BaF/3 cells transfected with hCXCR2. The specific conditions are described in the figure legends. The ND50 for the neutralization of myeloperoxidase release from human granulocytes is 0.5 - 1.5  $\mu$ g/mL in the presence of 1  $\mu$ g/mL rhGRO $\alpha$ .

Figure 1

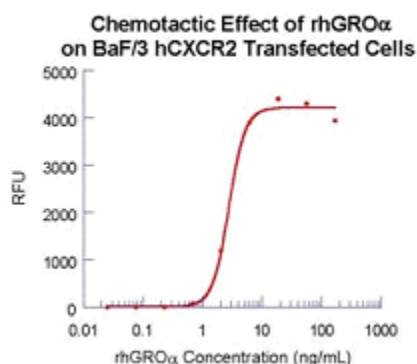


Figure 2

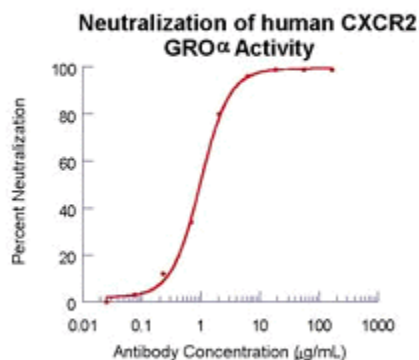


Figure 1

Human GRO $\alpha$  chemoattracts BaF/3 cells that have been transfected with hCXCR2. The number of cells that have migrated through to the lower chamber are quantitated using Resazurin Fluorescence staining. The ED50 for this effect is typically 1 - 4 ng/mL.

Figure 2

To measure the ability of the antibody to block rhGRO $\alpha$  mediated chemotaxis of BaF/3 hCXCR2 cells, rhGRO $\alpha$  at 5 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  $0.2 \times 10^6$  cells/well were added to the top wells of the chamber. After incubation for 3 hours at 37° C in a 5% CO<sub>2</sub> 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 ND50 for this lot of antibody is approximately 1 - 5  $\mu$ g/mL.