



Monoclonal Anti-human CXCL1/2/3/GRO Pan Specific Antibody

ORDERING INFORMATION

Catalog Number: MAB276

Clone: 31716

Lot Number: ZB01

Size: 500 µg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human CXCL1/2/3

Immunogen: *E. coli*-derived rhCXCL1/2/3

Ig class: mouse IgG₁

Recommended Applications:
Neutralization of bioactivity
Western blot

Other Application:
Direct ELISA

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, *E. coli*-derived, recombinant human CXCL1/2/3 (rhCXCL1/2/3). The IgG fraction of ascites fluid was purified by Protein A affinity chromatography. CXCL1, CXCL2 (MIP-2 α) and CXCL3 (MIP-2 β) are members of the alpha (C-X-C) subfamily of chemokines and are now designated GRO α , GRO β and GRO γ , respectively. Mature CXCL1/2/3 proteins bind with high affinity to the IL-8 receptor type B and are potent neutrophil attractants and activators.

Formulation

Lyophilized from a 0.2 µm sterile-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 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 biological activity of human CXCL1/2/3.

Applications

Neutralization of Human CXCL1/2/3 Bioactivity - The exact concentration of antibody required to neutralize human CXCL1/2/3 activity is dependent on the cytokine concentration, cell type, CXCL1/2/3 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.

As shown in figures 1 and 2 on the next page, the ND₅₀ for this lot of anti-hCXCL1/2/3 antibody was determined to be approximately 1 µg/mL, 0.4 µg/mL and 0.2 µg/mL for rhCXCL1, rhCXCL2 and rhCXCL3, respectively, using human CXCR2 transfected BaF/3 cells in a chemotaxis assay. The specific conditions are described in the figure legends.

Western Blot - This antibody can be used at 1 - 2 µg/mL with the appropriate secondary reagents to detect human CXCL1/2/3. The detection limit for rhCXCL2 is approximately 5 ng/lane under non-reducing and reducing conditions. The detection limit for rhCXCL1 is approximately 100 ng/lane and 50 ng/lane under non-reducing and reducing conditions, respectively. The detection limit for rhCXCL3 is approximately 25 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 human CXCL1/2/3. The detection limit for rhCXCL2 is approximately 0.3 ng/well, and the detection limit for rhCXCL2 and rhCXCL3 is approximately 2.5 ng/well.

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

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1-800-343-7475

Figure 1

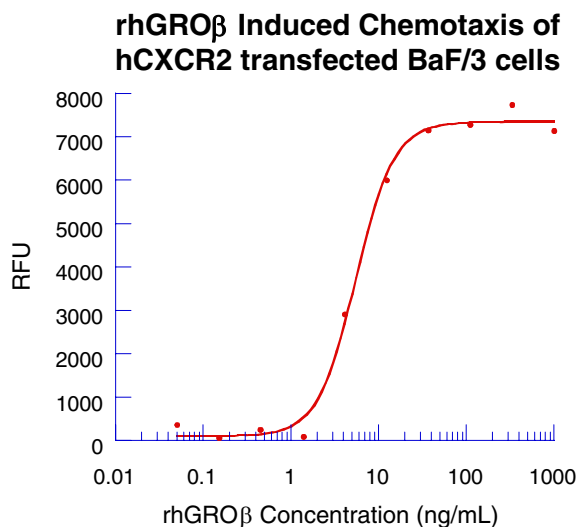


Figure 2

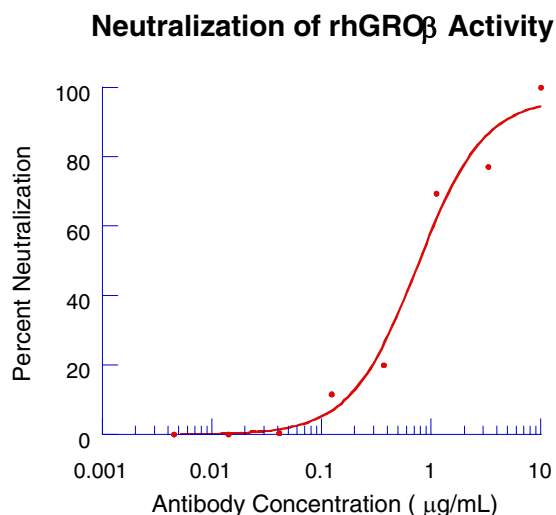


Figure 1

Human CXCL2 (R&D Systems, Catalog # 176-GB) can induce chemotaxis of mouse BaF/3 cells transfected with hCXCR2. The ED₅₀ for this effect is typically 1 - 5 ng/mL.

Figure 2

To measure the ability of the antibody to neutralize the chemoattractant activity of rhCXCL1/2/3 β for hCXCR2 transfected BaF/3 cells, rhCXCL2 (R&D Systems, Catalog # 176-GB) was incubated with various concentrations of the antibody for 30 minutes at room temperature in a 96 well microplate. Following this preincubation period, 75 μ L of the cytokine-antibody solution (containing rhCXCL2 at a final concentration of 6 ng/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 0.2×10^6 cells/well was added to the top chamber. After incubation for 3 hours at 37 $^{\circ}$ 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) overnight. The fluorescence was then read in a fluorescent microplate reader set at 544/590 nm. As shown in Figure 2, the ND₅₀ for this lot of antibody is approximately 0.15 - 0.75 μ g/mL.