



Monoclonal Anti-human CXCR3 Antibody

ORDERING INFORMATION

Catalog Number: MAB160

Clone: 49801

Lot Number: AOU07

Size: 500 µg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human CXCR3

Immunogen: CXCR3-transfected NS0 cells

Ig class: mouse IgG₁

Recommended Applications:

Neutralization of bioactivity
Flow cytometry
Immunohistochemistry

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse inoculated with human CXCR3-transfected NS0 mouse myeloma cells. The IgG fraction of ascites fluid was purified by Protein G affinity chromatography. CXCR3 is a G protein-coupled chemokine receptor that binds the α chemokines MIG (CXCL9), IP-10 (CXCL10) and I-TAC (CXCL11). CXCR3 is expressed on activated T cells, B cells and NK cells.

Formulation

Lyophilized from a 0.2 µm 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 react specifically with human CXCR3 transfectants and not the parental cell line using FACS analysis. This antibody does not react with CXCR1, CXCR2, CXCR4 or transfectants.

Applications

Neutralization of Human cell surface CXCR3 Mediated bioactivity - The exact concentration of antibody required to neutralize human cell surface CXCR3 activity is dependent on the ligand concentration as well as the number of CXCR3 receptors present on the cell surface (a function of cell type and culture conditions). 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 cell surface CXCR3 mediated rhl-TAC response on a responsive cell line, at a specific rhl-TAC concentration.

As shown in figures 1 and 2 on the next page, the ND₅₀ for this lot of anti-human CXCR3 antibody was determined to be approximately 1 - 6 µg/mL in the presence of 7 ng/mL of rhl-TAC, using the hCXCR3 transfected BaF/3 cells in a chemotaxis assay. The specific conditions are described in the figure legends.

Flow cytometry - Dilute this antibody to 5 - 10 µg/mL and add 10 µL of the diluted solution to 1 - 2.5 x 10⁵ cells in a total reaction volume not exceeding 200 µL. The binding of unlabeled monoclonal antibodies may be visualized by adding 10 µL of a 25 µg/mL stock solution of a secondary developing reagent such as goat anti-mouse IgG conjugated to a fluorochrome.

Immunohistochemistry - This antibody was used at a concentration of 10 µg/mL to detect CXCR3 in human tonsil. Cells were fixed with PBS containing 4% paraformaldehyde and blocked with PBS containing 10% normal donkey serum, 0.1% Triton[®] X-100, and 1% BSA. After blocking, cells were incubated with diluted primary antibody followed by NL557-coupled anti-mouse IgG in the dark. Between each step, cells were washed with PBS containing BSA.

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

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1-800-343-7475

Figure 1

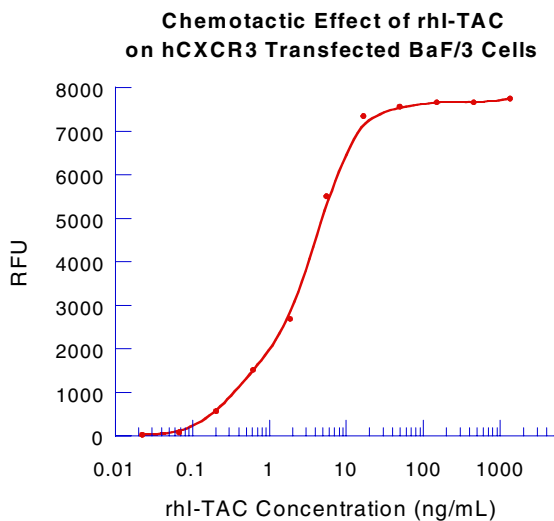


Figure 2

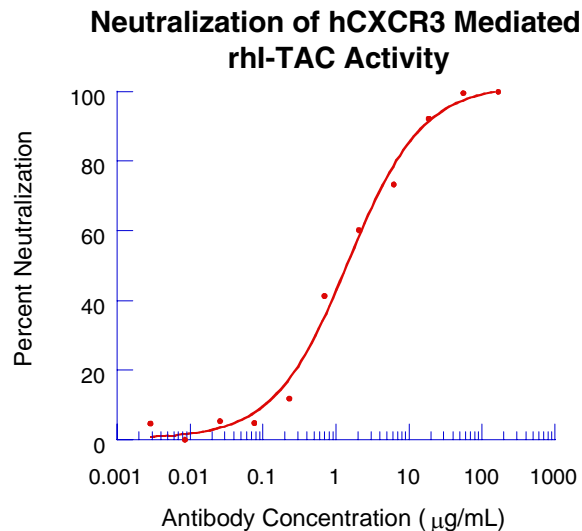


Figure 1

Human I-TAC chemoattracts hCXCR3 transfected BaF/3 cells. The number of cells that have migrated through to the lower chamber are quantitated using Resazurin fluorescence (R&D Systems, Catalog # AR002). The ED₅₀ for this effect is typically 1 - 5 ng/mL.

Figure 2

To measure the ability of the antibody to block rhI-TAC induced chemotaxis of hCXCR3 transfected BaF/3 cells, rhI-TAC at 7 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 Fluorescence. As shown in Figure 2, the ND₅₀ for this lot of antibody is approximately 1 - 6 µg/mL.