Monoclonal Anti-feline CXCL8/IL-8 Antibody

ORDERING INFORMATION

Catalog Number: MAB22772

Clone: 344615

Lot Number: ZTC01

Size: 500 μg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: feline CXCL8

Immunogen: *E. coli*-derived rfeCXCL8

Ig class: rat IgG_{2A}

Recommended Applications:
ELISA capture
Neutralization of bioactivity

Background

CXCL8, also known as IL-8, is a chemoattractant that recruits neutrophils to sites of inflammation, activates neutrophil functions and promotes angiogenesis. CXCL8 production is rapidly induced by pro-inflammatory signals in many different cell types. The biological activites of CXCL8 are mediated by chemokine receptors CXCR1 and CXCR2. Feline CXCL8 is translated as a 101 aa precursor protein with a 22 aa signal peptide. It shares 61% and 76% aa sequence identity with human and canine CXCL8, respectively.

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 purified, *E. coli*-derived, recombinant feline CXCL8 (rfeCXCL8; aa 23 - 101; Accession # Q9XSX5). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography.

Formulation

Lyophilized from a 0.2 μm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

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 detects rfeCXCL8 in direct ELISAs.

Application

ELISA capture - This product can be used as a capture reagent in a feline CXCL8 sandwich immunoassay in combination with biotinylated, feline CXCL8 detection antibody (Cat. # BAM22771) and recombinant feline CXCL8 (Cat. # 2277-FL) as the standard. The suggested coating concentration range is 2 - 8 μg/mL and should be titrated to determine the optimal concentration. A general protocol is provided at www.RnDSystems.com/go/MAPELISA. In this format, no cross-reactivity was observed with rhCXCL8, rcaCXCL8, or rpCXCL8.

Neutralization of feline CXCL8 bioactivity - The exact concentration of antibody required to neutralize rfeCXCL8 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 $Dose_{50}$ (ND_{50})** 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 ND_{50} for this lot of anti-feline CXCL8 antibody was determined to be approximately 0.075 – 0.375 μg/mL in the presence of 20 ng/mL of rfeCXCL8, using chemotaxis of mouse BaF/3 cells transfected with hCXCR2 as the bioassay. The specific conditions are described in the figure legends.

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

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1-800-343-7475

Figure 1

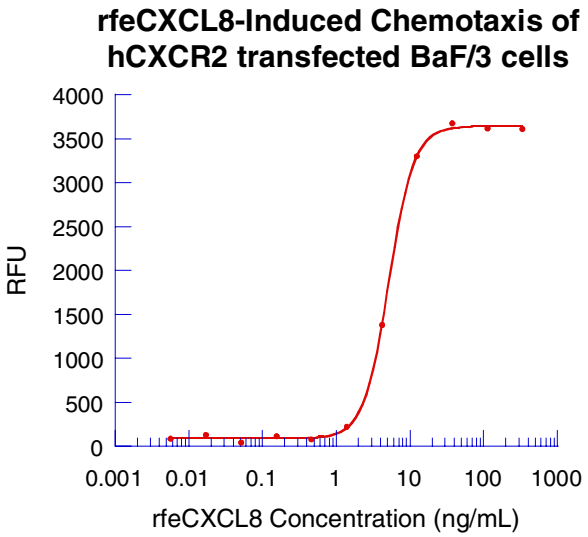

Figure 2

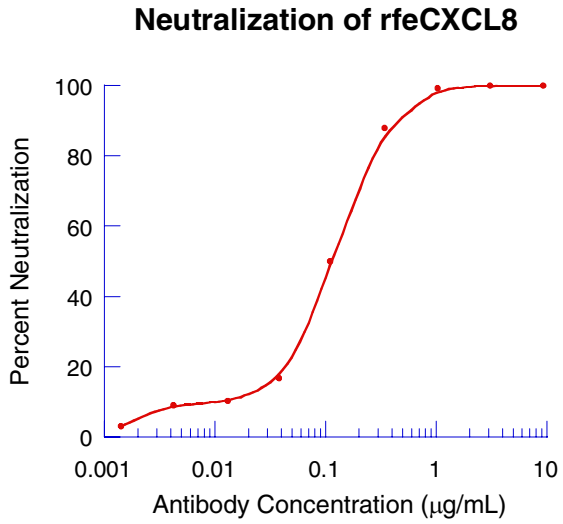

Figure 1
Feline CXCL8 can induce chemotaxis of mouse BaF/3 cells transfected with hCXCR2. The ED_{50} for this effect is typically 2 - 10 ng/mL.

Figure 2
To measure the ability of the antibody to neutralize the chemoattractant activity of rfeCXCL8 for BaF/3 cells transfected with hCXCR2 cells, rfeCXCL8 was incubated with various concentrations of the antibody for 30 minutes at room temperature in a 96 well microplate. Following this preincubation period, 75 μL of the cytokine-antibody solution (containing rfeCXCL8 at a final concentration of 20 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 x 10^6 cells/well were added to the top 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 (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 ND_{50} for this lot of antibody is approximately 0.075 - 0.375 μg/mL.