Anti-canine CXCL8/IL-8 Antibody

ORDERING INFORMATION

Catalog Number: AF1608

Lot Number: KDN01

Size: 100 μg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: canine CXCL8

Immunogen: *E. coli*-derived rcaCXCL8

Ig Type: goat IgG

Applications: Neutralization of bioactivity
Western blot
Direct ELISA
Immunocytochemistry

Preparation
Produced in goats immunized with purified, *E. coli*-derived, recombinant canine CXCL8 (rcaCXCL8). Canine CXCL8 specific IgG was purified by canine CXCL8 affinity chromatography.

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 0.5 mL of PBS is used, the antibody concentration will be 0.2 mg/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 has been selected for its ability to neutralize canine CXCL8 bioactivity.

Neutralization of Canine Interleukin 8 Bioactivity
The exact concentration of antibody required to neutralize rcaCXCL8 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.

As shown in figures 1 and 2 on the next page, the ND$_{50}$ for this lot of anti-canine CXCL8 antibody was determined to be approximately 0.1 - 0.5 μg/mL in the presence of 10 ng/mL of caCXCL8, using chemotaxis of hCXCR2 transfected BaF/3 cells as the bioassay. The specific conditions and experimental details are described in the figure legends.

Additional Applications
Western blot - This antibody can be used at 0.1 - 0.2 μg/mL with the appropriate secondary reagents to detect canine CXCL8. The detection limit for rcaCXCL8 is approximately 5 ng/lane under non-reducing and reducing conditions. In this format, this antibody shows less than 1% cross-reactivity with rhCXCL8, rpCXCL8, rrCINC-1, rrCINC-2α and rrCINC-2β.

Direct ELISA - This antibody can be used at 0.5 - 1.0 μg/mL with the appropriate secondary reagents to detect canine CXCL8. The detection limit for rcaCXCL8 is approximately 0.8 ng/well.

Immunocytochemistry - This antibody will detect CXCL8 in cells. The working dilution is 5 - 15 μg/mL. For chromogenic detection of labeling, use R&D Systems' Cell and Tissue Staining Kits (CTS Series).

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

R&D Systems, Inc.
1-800-343-7475

Figure 1

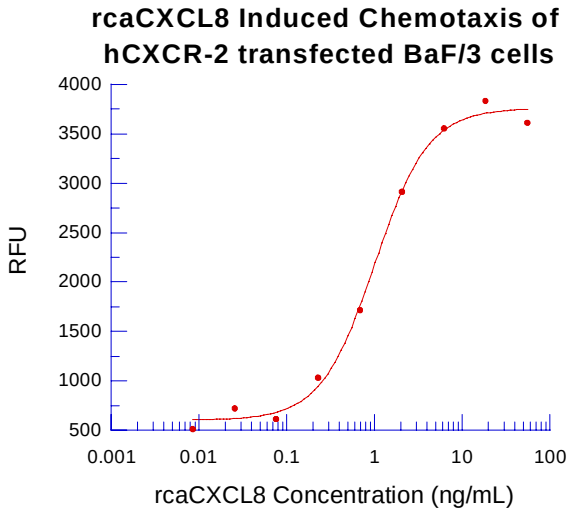

Figure 2

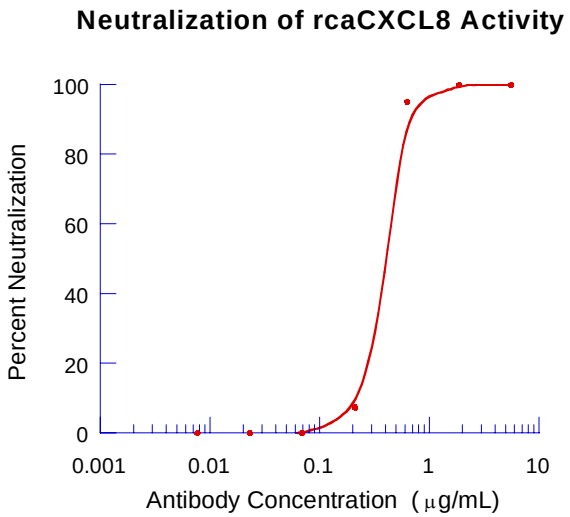

Figure 1
Canine CXCL8 can induce chemotaxis of mouse BaF/3 cells transfected with hCXCR2. The ED_{50} for this effect is typically 0.15 - 0.75 ng/mL.

Figure 2
To measure the ability of the antibody to neutralize the chemoattractant activity of rcaCXCL8 for hCXCR2 transfected BaF/3 cells, rhCXCL8 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 rcaCXCL8 at a final concentration of 10 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 was 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 0.1 - 0.5 μg/mL.