Anti-rat CINC-2α/β Antibody

ORDERING INFORMATION

Catalog Number: AF516

Lot Number: BIB02

Size: 100 μg

Formulation: 0.2 μm filtered solution in PBS

Storage: -20° C

Reconstitution: sterile PBS

Specificity: rrCINC-2α and CINC-2β

Immunogen: *E. coli*-derived rrCINC-2α

Ig Type: rat CINC-2α and CINC-2β specific goat IgG

Applications: Neutralization of bioactivity
Western blot
ELISA

Preparation
Produced in goats immunized with purified, *E. coli*-derived, recombinant rat cytokine-induced neutrophil chemoattractant 2 alpha (rrCINC-2α). CINC-2α/β specific IgG was purified by rat CINC-2α affinity chromatography.

Formulation
Lyophilized from a 0.2 μm filtered solution in phosphate-buffered saline (PBS).

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 0.1 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 rrCINC-2α bioactivity. Based on direct ELISA results, this antibody shows greater than 50% cross-reactivity with rrCINC-1, rrCINC-2β, rrCINC-3, less than 25% cross-reactivity with rmKC, rmMIP-2, rhGROα, rhGROβ and rhGROγ and less than 10% cross-reactivity with rhIL-8. Additionally, in direct ELISA, this antibody shows no cross-reactivity with other chemokines tested.[1]

Neutralization of Rat CINC-2α Bioactivity
The exact concentration of antibody required to neutralize rrCINC-2α 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-rat CINC-2α antibody was determined to be approximately 0.5 - 2 μg/mL in the presence of 0.05 μg/mL of rrCINC-2α, using BaF/3 cells transfected with rhCXCR-2. The specific conditions are described in the figure legends.

Additional Applications
Direct ELISA - This antibody can be used at 0.5 - 1.0 μg/mL with the appropriate secondary reagents to detect rat CINC-2α. The detection limit for rrCINC-2α is approximately 0.01 ng/well.

Western blot - This antibody can be used at 0.1 - 0.2 μg/mL with the appropriate secondary reagents to detect rat CINC-2α. The detection limit forrrCINC-2α is approximately 2 ng/lane and 20 ng/lane under non-reducing and reducing conditions, respectively.

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

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1-800-343-7475

Figure 1

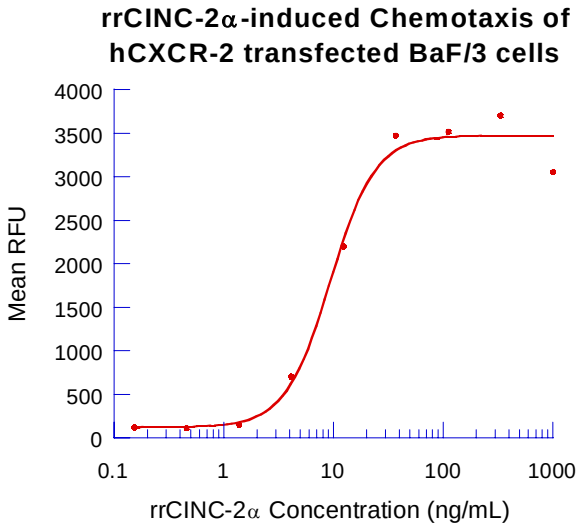

Figure 2

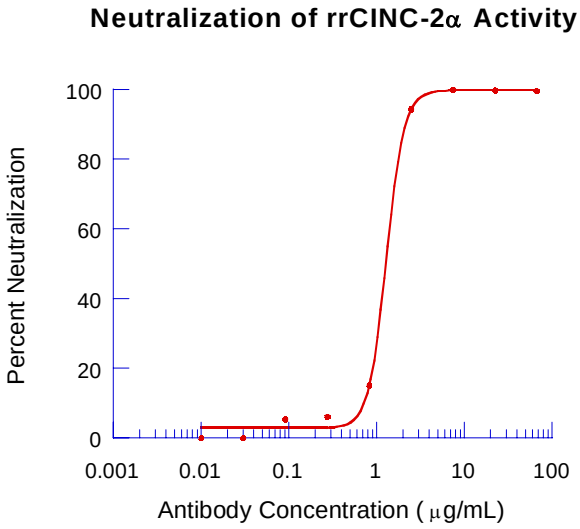

Figure 1
Recombinant rat CINC-2α chemoattracts BaF/3 cells transfected with rhCXCR-2. The ED_{50} for this effect is typically 4 - 20 ng/mL.

Figure 2
To measure the ability of the antibody to neutralize the chemoattractant activity of rrCINC-2α for hCXCR-2 transfected BaF/3 cells, rrCINC-2α was incubated with various concentrations of the antibody for 15 minutes at room temperature in a 96 well microplate. Following this preincubation period, 75 μL of the cytokine-antibody solution (containing rrCINC-2α at a final concentration of 0.05 μg/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 carefully disassembled. The cells that migrate through to the lower chamber were transferred to a 96 well plate. Chemotaxis was measued by Resazurin (R&D Systems, Catalog # AR002) staining of cells that have migrated through the filter. As shown in figure 2, the ND_{50} for this lot of antibody is approximately 0.5 - 2 μg/mL.