



Anti-canine IL-4 Antibody

ORDERING INFORMATION

Catalog Number: AF754

Lot Number: FBM02

Size: 100 µg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: canine IL-4

Immunogen: *E. coli*-derived rcaIL-4

Ig Type: canine IL-4 specific goat IgG

Applications: Neutralization of bioactivity
Direct ELISA
Western blot

Preparation

Produced in goats immunized with purified, *E. coli*-derived, recombinant canine interleukin 4 (rcaIL-4). Canine IL-4 specific IgG was purified by canine IL-4 affinity chromatography.

Formulation

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

Endotoxin Level

< 0.01 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 rcaIL-4 bioactivity.

Neutralization of Canine IL-4 Bioactivity

The exact concentration of antibody required to neutralize rcaIL-4 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 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.

The ND₅₀ for this lot of anti-canine IL-4 antibody was determined to be approximately 2 - 8 µg/mL in the presence of 50 ng/mL of rcaIL-4, using the human TF-1 cell line. 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 canine IL-4. The detection limit for rcaIL-4 is approximately 3 ng/well. In this format, this antibody shows approximately 40% cross-reactivity with rhIL-4, 15% cross-reactivity with rfEL-4 and rpIL-4 and less than 1% cross-reactivity with rcIL-4, rmIL-4 and rrIL-4.

Western blot - This antibody can be used at 0.1 - 0.2 µg/mL with the appropriate secondary reagents to detect canine IL-4. The detection limit for rcaIL-4 is approximately 5 ng/lane under non-reducing and reducing conditions.

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

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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

Figure 1

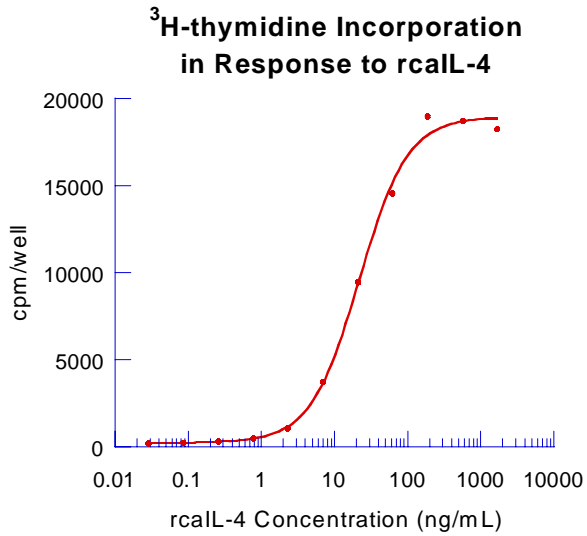


Figure 2

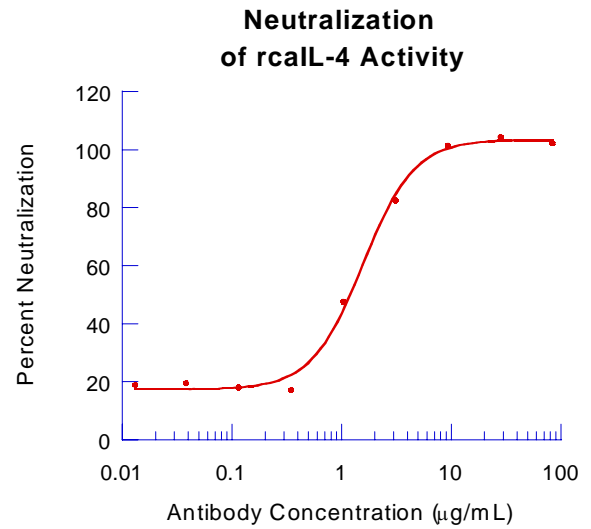


Figure 1

Canine IL-4 stimulates the ³H-thymidine incorporation by TF-1 cells in a dose-dependent manner (Kitamura, T. *et al.*, 1989, *J. Cell Physiol.* **140**(2):232-333). The ED₅₀ for this effect is typically 10 - 40 ng/mL.

Figure 2

To measure the ability of the antibody to neutralize the bioactivity of rcaIL-4 on human TF-1 cells, rcaIL-4 was incubated with various concentrations of the antibody for 1 hour at 37° C in a 96 well plate. Following this preincubation period, TF-1 cells were added. The assay mixture in a total volume of 100 µL, containing antibody at the concentrations indicated, rcaIL-4 at 50 ng/mL and cells at 1 x 10⁵ cells/mL, was incubated at 37° C for 48 hours in a humidified CO₂ incubator. ³H-thymidine was added during the final 4 hours of incubation. The cells were subsequently harvested onto glass fiber filters and the ³H-thymidine incorporated into DNA was determined. The ND₅₀ of the antibody is approximately 2 - 8 µg/mL.