



Anti-mouse IL-3 Antibody

ORDERING INFORMATION

Catalog Number: AB-403-NA

Lot Number: BS03

Size: 1 mg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: mouse IL-3

Immunogen: *E. coli*-derived rmlL-3

Ig class: goat IgG

Applications: Neutralization of bioactivity
Western blot
Direct ELISA

Preparation

This antibody was produced in goats immunized with purified, *E. coli*-derived, recombinant mouse interleukin 3 (rmlL-3). Total IgG was purified by Protein G 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 1 mL of PBS is used, the antibody concentration will be 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 the biological activity of rmlL-3. It will not neutralize the biological activity of rhIL-3. In direct ELISA, this antibody shows less than 5% cross-reactivity with rrIL-3 and less than 1% cross-reactivity with rhIL-3.

Neutralization of Mouse IL-3 Bioactivity

The exact concentration of antibody required to neutralize rmlL-3 activity is dependent on the biological effect studied, the cell type, and incubation conditions. To provide a guideline, R&D Systems has determined the neutralization dose within a specific cell type for each of its antibodies.

Neutralization Dose₅₀ (ND₅₀) - that concentration of antibody required to yield one-half maximal inhibition of the cytokine, when that cytokine is present at five times its normal ED₅₀ (a concentration of five times the ED₅₀ will normally yield 100% activity; figure 1, see page two). The ND₅₀ can be used to calculate the amount of antibody needed in a particular application.

The ND₅₀ for this lot of anti-mouse IL-3 was determined to be approximately 0.015 - 0.025 µg/mL in a neutralizing bioassay using the factor dependent murine cell line, NFS-60. Results of this assay are seen in figure 2 (see page two).

In these experiments, rmlL-3 was pre-incubated with increasing concentrations of antibody for 1 hour at 37° C in a 96 well microplate. Following this preincubation period, NFS-60 cells were added to give a final concentration of 5 x 10⁴ cells/mL. The assay mixture, in a total volume of 200 µL/well, containing rmlL-3 at a final concentration of 0.5 ng/mL and antibody at the concentrations indicated, was incubated for 24 hours at 37° C in a 5% CO₂ humidified incubator and pulsed with ³H-thymidine for the final 4 hours. The contents of the wells were harvested onto glass fiber filters, and the ³H-thymidine incorporated into DNA was determined.

Additional Applications

Western blot - This antibody can be used at 1 - 2 µg/mL with the appropriate secondary reagents to detect mouse IL-3. The detection limit for rmlL-3 is approximately 1 ng/lane under non-reducing and reducing conditions.

Direct ELISA - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect mouse IL-3. The detection limit for rmlL-3 is approximately 1 ng/well.

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

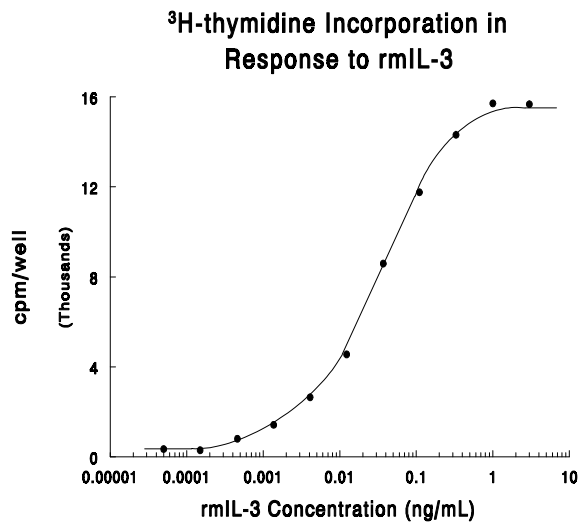
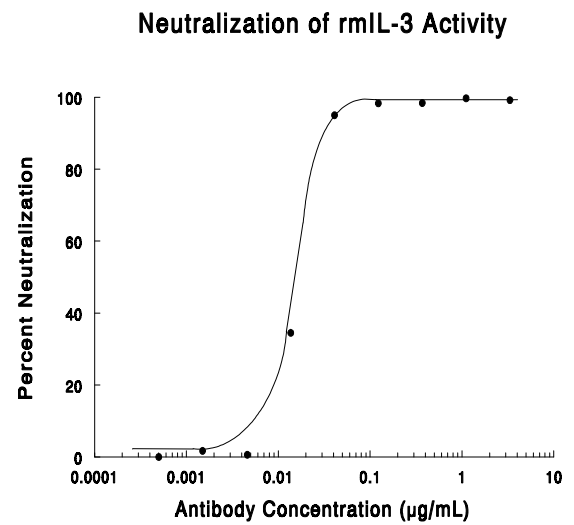


Figure 2



The biological activity of rmlL-3 was measured in a cell proliferation assay using the factor dependent cell line, NFS-60 (Holmes, K.L. *et al.*, 1985, Proc. Natl. Acad. Sci. USA **82**:6687). **Figure 1** shows that NFS-60 cell proliferation, as measured by ³H-thymidine incorporation, is dependent on rmlL-3. The ED₅₀ for this effect is approximately 0.05 - 0.1 ng/mL of rmlL-3. Assuming a nominal ED₅₀ of 0.1 ng/mL, five times the ED₅₀ of rmlL-3 (0.5 ng/mL) was used to assess the neutralizing activity of this lot of antibody. As seen in **figure 2**, the ND₅₀ for this lot of antibody is approximately 0.015 - 0.025 µg/mL.