

ORDERING INFORMATION

Catalog Number: MAB5452

Clone: 299298

Lot Number: XCL01

Size: 100 µg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: rat IL-5

Immunogen: Sf21-derived rIL-5

Ig class: mouse IgG₁

Recommended Applications:
Neutralization of bioactivity
Immunohistochemistry

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, Sf21-derived recombinant rat interleukin 5 (rrIL-5). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. IL-5 is a disulfide-linked homodimeric cytokine that is secreted by T cells. IL-5 promotes the proliferation, differentiation and activation of eosinophils. It binds to a receptor complex consisting of one IL-5 specific α chain and one non-binding common β chain that is shared with the receptors for GM-CSF and IL-3.

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 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 rat IL-5 in immunohistochemistry.

Applications

Neutralization of Rat IL-5 Bioactivity -The exact concentration of antibody required to neutralize rat IL-5 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-rat IL-5 antibody was determined to be approximately 0.2 - 1 µg/mL in the presence of 1.25 ng/mL of rIL-5, measuring proliferation of the human factor-dependent cell line, TF-1, as an assay. The specific conditions are described in the figure legends.

Immunohistochemistry - This antibody was used at a concentration of 25 µg/mL with appropriate secondary reagents to detect IL-5 in paraformaldehyde-fixed rat splenocytes. For chromogenic detection of labeling, the use of R&D Systems Cell and Tissue Staining Kits (CTS Series) is recommended.

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

For immunohistochemistry images, please refer to our website at
<http://www.rndsystems.com/go/ihc>

Figure 1

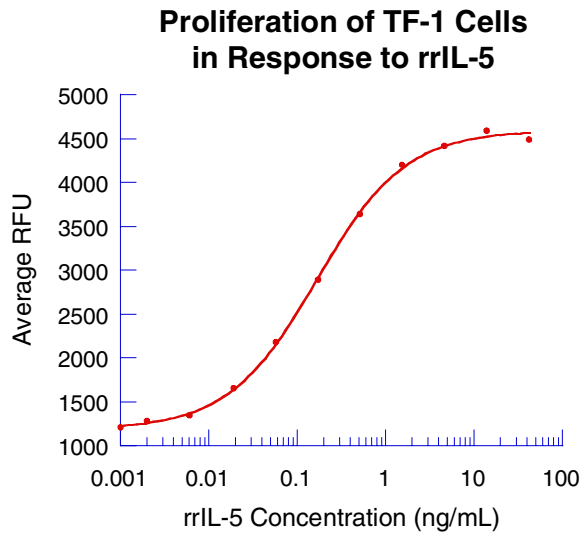


Figure 2

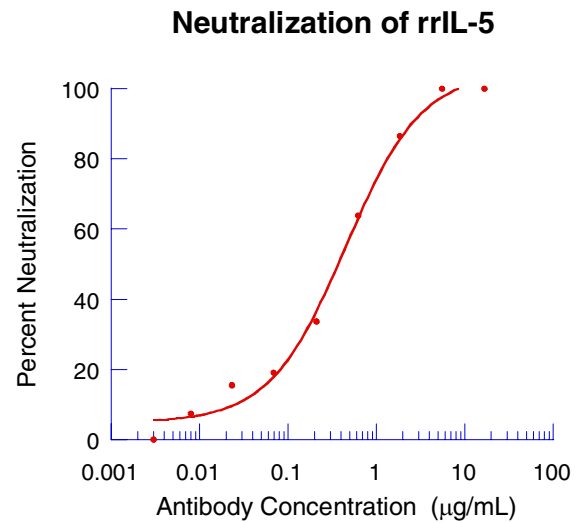


Figure 1

Rat IL-5 stimulates the proliferation of TF-1 cells in a dose-dependent manner (Kitamura, T. *et al.*, 1989, *J. Cell Physiol.* **140**:323 - 333). The ED₅₀ for this effect is typically 0.05 - 0.2 ng/mL.

Figure 2

To measure the ability of the antibody to neutralize the bioactivity of rrIL-5 on human TF-1 cells, rrIL-5 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, rrIL-5 at 1.25 ng/mL and cells at 1 x 10⁵ cells/mL, was incubated at 37° C for 48 hours in a humidified CO₂ incubator. Resazurin was added during the final 16 - 20 hours of incubation to measure cell growth. The relative fluorescence was then read in a fluorescent plate reader set at Ex. 544/Em. 590. The ND₅₀ of the antibody is approximately 0.2 - 1 µg/mL.