Monoclonal Anti-cotton rat IL-6 Antibody

ORDERING INFORMATION

Catalog Number: MAB561

Clone: 147135

Lot Number: FXE03

Size: 500 μg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: cotton rat IL-6

Immunogen: *E. coli*-derived rcrIL-6

Ig class: mouse IgG_{2A}

Recommended Applications:
Neutralization of bioactivity
Western blot

Other Application:
Direct ELISA

Background
IL-6 is a multifunctional protein that plays important roles in host defense, acute phase reactions, immune responses, and hematopoiesis. IL-6 is produced by various lymphoid and nonlymphoid cells. Its effects are mediated by the dimeric receptor complex composed of IL-6 R and gp130.

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, *E. coli*-derived, recombinant cotton rat interleukin 6 (rcrIL-6; aa 25 - 212; Accession # AAL18819). The IgG fraction of the tissue culture supernatant 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 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 rcrIL-6 in direct ELISAs and Western blots. In direct ELISAs, this antibody showed no cross-reactivity with other cytokines tested.[1]

Applications
Neutralization of Cotton Rat IL-6 bioactivity - The exact concentration of antibody required to neutralize rcrIL-6 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-cotton rat IL-6 antibody was determined to be approximately 3 - 15 μg/mL in the presence of 0.1 ng/mL of rcrIL-6. The rcrIL-6-dependent proliferation of T1165.85.2.1 cells was used as an assay. The specific conditions are described in the figure legends.

Western blot - This antibody can be used at 1 - 2 μg/mL with the appropriate secondary reagents to detect cotton rat IL-6. Using a colorimetric detection system, the detection limit for rcrIL-6 is approximately 50 ng/lane under non-reducing and reducing conditions. Chemiluminescent detection will increase sensitivity by 5 to 50 fold.

Direct ELISA - This antibody can be used at 0.5 - 1.0 μg/mL with the appropriate secondary reagents to detect cotton rat IL-6. The detection limit for rcrIL-6 is approximately 15 ng/well.

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

1. rmIL-6, rhIL-6, rrIL-6, rpIL-6, rhCLC, rrCNTF, rmCT-1, rmIL-11, rmLIF and rmOSM.

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

Figure 1

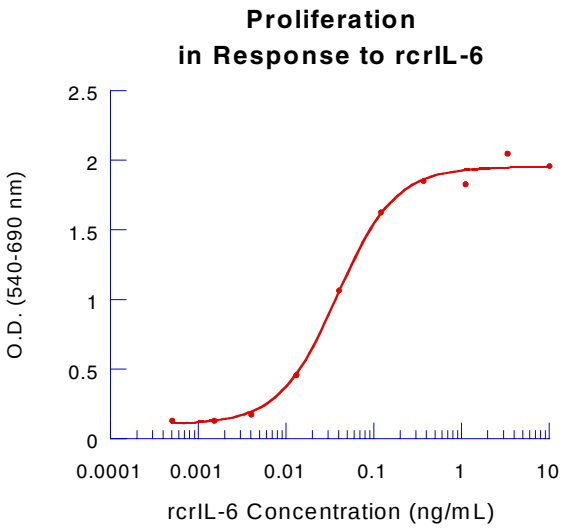

Figure 2

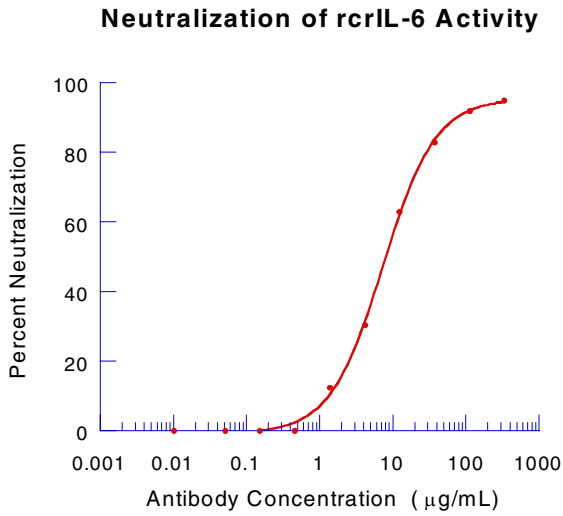

Figure 1
Cotton rat IL-6 stimulates the proliferation of the IL-6 dependent murine plasmacytoma cells, T1165.85.2.1 in a dose-dependent manner (Nordan, R.P. and M. Potter, 1986, Science **233**:566 - 569). The ED_{50} for this effect is typically 0.02 - 0.06 ng/mL.

Figure 2
To measure the ability of the antibody to neutralize the bioactivity of rcrIL-6 on T1165.85.2.1 cells, rcrIL-6 was incubated with various concentrations of the antibody for 1 hour at 37° C in a 96 well microplate. Following this preincubation period, T1165.85.2.1 cells were added. The assay mixture in a total volume of 200 µL, containing antibody at the concentrations indicated, rcrIL-6 at 0.1 ng/mL and cells at 1 x 10^5 cells/mL, was incubated at 37° C for 48 hours in a humidified CO_2 incubator. MTT was added during the final 4 hours of incubation. The crystallized dye was subsequently solubilized and the optical density was read on the ELISA plate reader set to 540 nm. The ND_{50} of the antibody is approximately 3 - 15 µg/mL.