



Anti-equine IL-10 Antibody

ORDERING INFORMATION

Catalog Number: AF1605

Lot Number: UAQ01

Size: 100 µg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: equine IL-10

Immunogen: *E. coli*-derived reqIL-10

Ig Type: goat IgG

Applications: Neutralization of bioactivity
Western blot
Immunocytochemistry
ELISA capture

Preparation

Produced in goats immunized with purified, *E. coli*-derived, recombinant equine interleukin 10 (reqIL-10; R&D Systems, Catalog # 1605-IL). Equine IL-10 specific IgG was purified by equine IL-10 affinity chromatography.

Formulation

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

Endotoxin Level

< 0.15 EU per 1 µg of the antibody as determined by the LAL method.

Reconstitution

Reconstitute with sterile PBS. If 0.5 mL of PBS is used, the antibody concentration will be 0.2 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 equine IL-10 bioactivity.

Neutralization of Equine IL-10 bioactivity

The exact concentration of antibody required to neutralize equine IL-10 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-equine IL-10 antibody was determined to be approximately 0.2 - 0.6 µg/mL in the presence of 20 ng/mL of reqIL-10, using MC/9-2 cells. The specific conditions are described in the figure legends.

Additional Applications

Western blot - This antibody can be used at 0.1 - 0.2 µg/mL with the appropriate secondary reagents to detect equine IL-10. The detection limit for reqIL-10 is approximately 5 ng/lane and 2 ng/lane under non-reducing and reducing conditions, respectively.

Immunocytochemistry - This antibody will detect IL-10 in cells. The working dilution is 15 µg/mL. For chromogenic detection of labeling, use R&D Systems Cell and Tissue Staining Kits (CTS Series).

ELISA capture - This product can be used as a capture reagent in a equine IL-10 sandwich immunoassay in combination with biotinylated equine IL-10 detection antibody (Cat. # BAF1605) and recombinant equine IL-10 (Cat. # 1605-IL) as the standard. The suggested coating concentration range is 0.2 - 0.8 µg/mL and should be titrated to determine the optimal concentration. A general protocol is provided at www.RnDSystems.com/MAPELISA. In this format, less than 1% cross-reactivity is observed with rcalL-10 and rpIL-10, and less than 0.4% cross-reactivity is observed with rhIL-10, rmlL-10, rrlL-10 and rfelL-10.

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

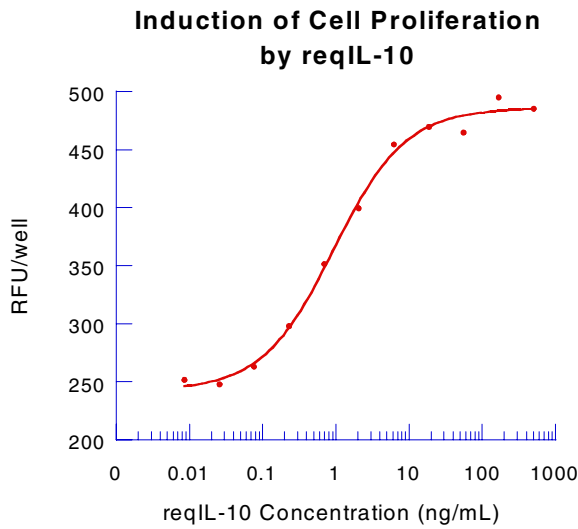


Figure 2

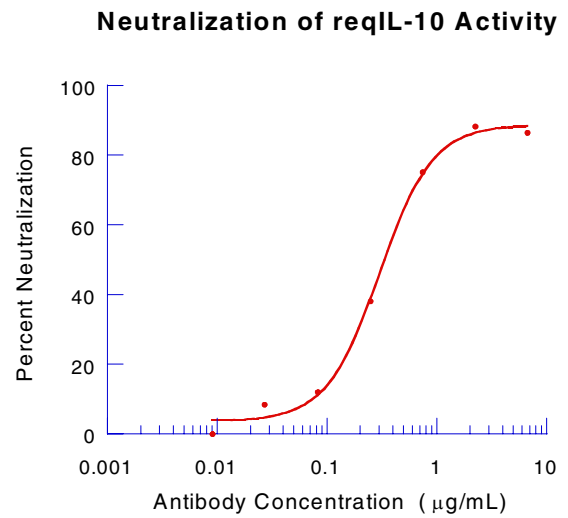


Figure 1

Recombinant equine IL-10 stimulates proliferation of MC/9-2 cells in a dose-dependent manner. Resazurin (R&D Systems, Catalog # AR002) was added during the final 16 - 20 hours of incubation to quantitate cell growth. The ED₅₀ for this effect is typically 1 - 3 ng/mL.

Figure 2

To measure the ability of the antibody to neutralize the bioactivity of reqIL-10 on MC/9-2 cells, reqIL-10 was incubated with various concentrations of the antibody for 1 hour at 37° C in a 96 well plate. Following this preincubation period, MC/9-2 cells were added. The assay mixture, in a total volume of 100 µL, containing antibody at the concentrations indicated, reqIL-10 at 20 ng/mL and cells at 1 x 10⁵ cells/mL was incubated at 37° C for 48 - 72 hours in a humidified CO₂ incubator. Resazurin (R&D Systems, Catalog # AR002) was added during the final 4 hours of incubation to quantitate 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 - 0.6 µg/mL.