



# Anti-equine IL-6 Antibody

## ORDERING INFORMATION

**Catalog Number:** AF1886

**Lot Number:** KWP01

**Size:** 100 µg

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

**Storage:** -20° C

**Reconstitution:** sterile PBS

**Specificity:** equine IL-6

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

**Ig Type:** goat IgG

**Applications:** Neutralization of bioactivity  
Western blot  
Direct ELISA

## Preparation

Produced in goats immunized with purified, *E. coli*-derived, recombinant equine interleukin 6 (reqIL-6). Equine IL-6 specific IgG was purified by equine IL-6 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 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-6 bioactivity. In direct ELISAs and western blots, this antibody shows approximately 50% cross-reactivity with rhIL-6, rpIL-6, rcaIL-6 and 20% cross-reactivity with rmIL-6 and rrIL-6.

## Neutralization of Equine IL-6 Bioactivity

The exact concentration of antibody required to neutralize reqIL-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<sub>50</sub> (ND<sub>50</sub>)** 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<sub>50</sub> for this lot of anti-equine IL-6 antibody was determined to be approximately 0.5 - 1.5 µg/mL in the presence of 4 ng/mL of reqIL-6, using the IL-6-dependent murine plasmacytoma cell line, T1165.85.2.1. 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 equine IL-6. The detection limit for reqIL-6 is approximately 1.2 ng/well.

**Western blot** - This antibody can be used at 0.1 - 0.2 µg/mL with the appropriate secondary reagents to detect equine IL-6. The detection limit for reqIL-6 is approximately 2 ng/lane under non-reducing and reducing conditions.

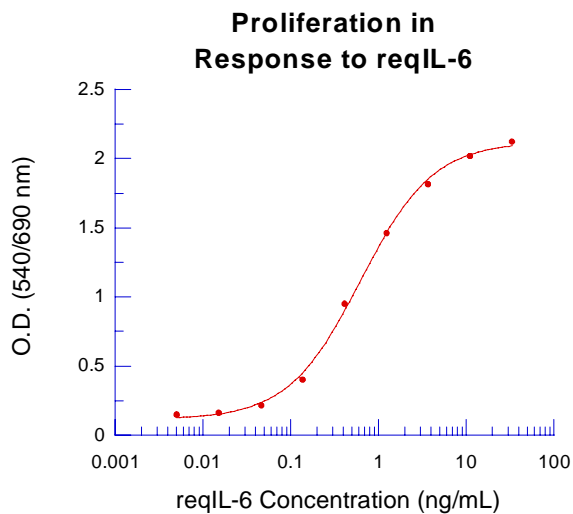
**Immunocytochemistry** - This antibody will detect IL-6 in cells. The working dilution is 15 µg/mL. For chromogenic detection of labeling, use R&D Systems' Cell and Tissue Staining Kits (CTS Series).

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

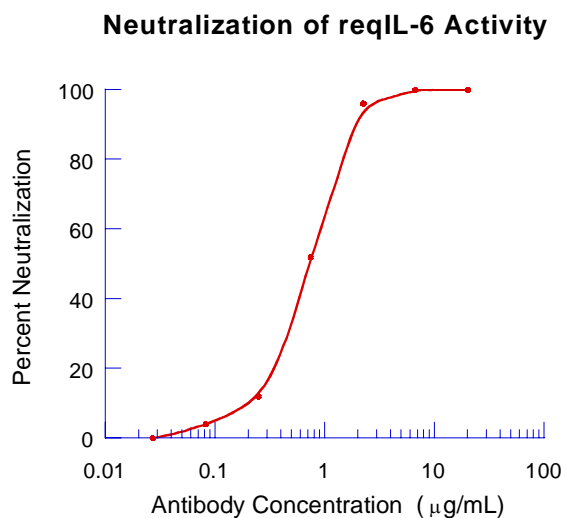
FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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

**Figure 1**



**Figure 2**



**Figure 1**

Equine 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<sub>50</sub> for this effect is typically 0.15 - 0.75 ng/mL.

**Figure 2**

To measure the ability of the antibody to neutralize the bioactivity of reqIL-6 on T1165.85.2.1 cells, reqIL-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, reqIL-6 at 4 ng/mL and cells at 1 x 10<sup>5</sup> cells/mL, was incubated at 37° C for 48 hours in a humidified CO<sub>2</sub> 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<sub>50</sub> of the antibody is approximately 0.5 - 1.5 µg/mL.