



Anti-equine IFN- γ Antibody

ORDERING INFORMATION

Catalog Number: AF1586

Lot Number: UGH01

Size: 100 μ g

Formulation: 0.2 μ m filtered solution in PBS with 5% trehalose

Storage: -20° C

Reconstitution: sterile PBS

Specificity: equine IFN- γ

Immunogen: *E. coli*-derived reqIFN- γ

Ig Type: goat IgG

Applications: Western blot
Immunocytochemistry
Neutralization of bioactivity
ELISA capture

Preparation

Produced in goats immunized with purified, *E. coli*-derived, recombinant equine interferon gamma (reqIFN- γ). Equine IFN- γ specific IgG was purified by equine IFN- γ affinity chromatography.

Formulation

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

Endotoxin Level

< 0.1 EU per 1 μ g 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 recognize equine IFN- γ in the applications listed below.

Applications

Western blot - This antibody can be used at 0.1 - 0.2 μ g/mL with the appropriate secondary reagents to detect equine, canine and feline IFN- γ . The detection limit for reqIFN- γ , rcalIFN- γ and rfelIFN- γ is approximately 2 ng/lane under non-reducing and reducing conditions.

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

Neutralization of Equine IFN- γ bioactivity - The exact concentration of antibody required to neutralize reqIFN- γ 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 IFN- γ antibody was determined to be approximately 0.25 - 1.25 μ g/mL in the presence of 20 ng/mL of reqIFN- γ , based on the anti-viral activity of reqIFN- γ . The specific conditions are described in the figure legends.

ELISA capture - This product can be used as a capture reagent in a equine IFN- γ sandwich immunoassay in combination with biotinylated equine IFN- γ detection antibody (Cat. # BAF1586) and recombinant equine IFN- γ (Cat. # 1586-HG) as the standard. The suggested coating concentration range is 0.2 - 0.8 μ g/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 20% cross-reactivity with rcalIFN- γ is observed, less than 6% cross-reactivity with rblIFN- γ and rfelIFN- γ is observed, and less than 0.2% cross-reactivity with rhIFN- γ , rmIFN- γ , rrIFN- γ , rpIFN- γ , rrmIFN- γ and rcrIFN- γ is observed.

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

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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1-800-343-7475

Figure 1

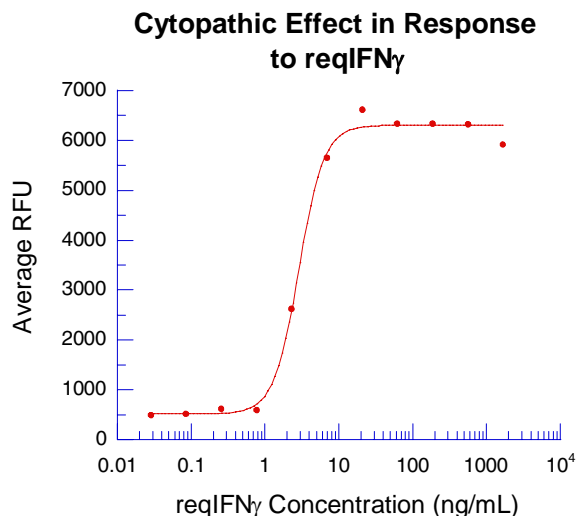
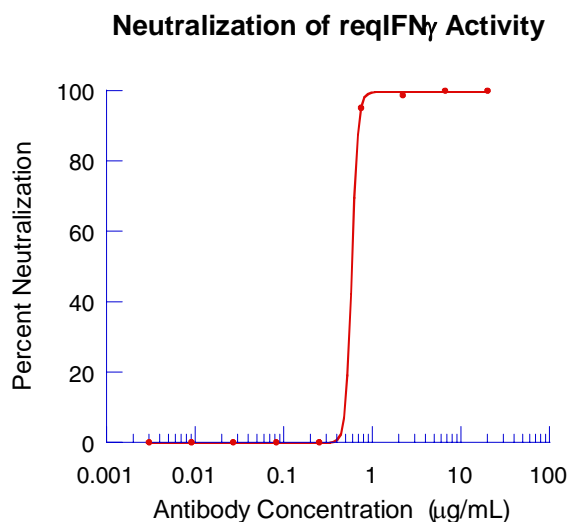


Figure 2



Typical Data

Figure 1

Equine IFN- γ reduces the cytopathic effect of the lytic virus EMC in a dose-dependent manner, on the mouse cell line, L-929 (Vogel, S. and M. Hogan, 1995, in *Current Protocols in Immunology*, R. Ciocio, ed.; John Wiley & Sons, Inc. p. 6.9.1). The ED₅₀ for this effect is typically 2 - 8 ng/mL.

Figure 2

To measure the ability of the antibody to neutralize the bioactivity of reqIFN- γ on L-929 cells, reqIFN- γ was added to various concentrations of the antibody. The antigen-antibody mixture was added to confluent cultures of L-929 cells in a 96 well plate. The assay mixture in a total volume of 100 μ L, containing antibody at the concentrations indicated and reqIFN- γ at 20 ng/mL, was incubated at 37 $^{\circ}$ C for 20 - 24 hours in a humidified CO₂ incubator. At the end of the incubation period, medium was aspirated from all wells and an appropriate titrated amount of Encephalomyocarditis virus (EMCV) in prewarmed culture medium was added to each test well. After an additional 20 - 24 hour incubation period, Resazurin (R&D Systems, Catalog # AR002) was added to the plate to measure the cytopathic effect of the EMC virus. After the 16 - 18 hour Resazurin incubation, the fluorescence was read in a fluorescent plate reader set at Ex. 544/590 nm. The ND₅₀ of the antibody is approximately 0.25 - 1.25 μ g/mL.