



Anti-human LAP (TGF- β 1) Antibody

ORDERING INFORMATION

Catalog Number: AF-246-NA

Lot Number: EF02

Size: 100 μ g

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human LAP (TGF- β 1)

Immunogen: CHO cell-derived rhLAP (TGF- β 1)

Ig Type: goat IgG

Applications: Neutralization of bioactivity
Western blot
Immunohistochemistry
Direct ELISA

Preparation

Produced in goats immunized with purified, CHO cell-derived, recombinant human TGF- β 1 latency associated peptide [rhLAP (TGF- β 1)]. LAP (TGF- β 1) specific IgG was purified by human LAP (TGF- β 1) 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 1 mL of PBS is used, the antibody concentration will be 0.1 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 the biological activity of rhLAP. Based on direct ELISA and western blot results, this antibody is specific for the LAP derived from the TGF- β 1 precursor, but does not react with LAP from the TGF- β 2 precursor.

Neutralization of Human LAP (TGF- β 1) bioactivity

The exact concentration of antibody required to neutralize LAP 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.

As shown in figures 1 and 2 on the next page, the ND₅₀ for this lot of anti-LAP antibody was determined to be approximately 0.05 - 0.15 μ g/mL in the presence of 125 ng/mL of rhLAP, using TGF- β responsive HT-2 cells as target cells. The specific conditions are described in the figure legends.

Additional Applications

Western blot - This antibody can be used at 0.1 - 0.2 μ g/mL with the appropriate secondary reagents to detect human LAP (TGF- β 1). The detection limit for rhLAP (TGF- β 1) is approximately 5.0 ng/lane under non-reducing and reducing conditions.

Immunohistochemistry - This antibody has been used successfully in immunohistochemistry applications for the detection of latent TGF- β 1 (Ewan, K.B. *et al.*, (2002) *Am. J. Pathol.* 2081 - 93).

Direct ELISA - This antibody can be used at 0.5 - 1.0 μ g/mL with the appropriate secondary reagents to detect human LAP (TGF- β 1). The detection limit for rhLAP (TGF- β 1) is approximately 0.31 ng/well.

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

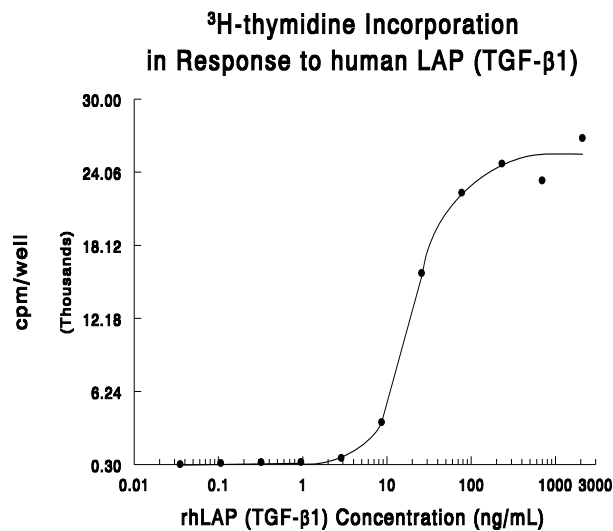


Figure 2

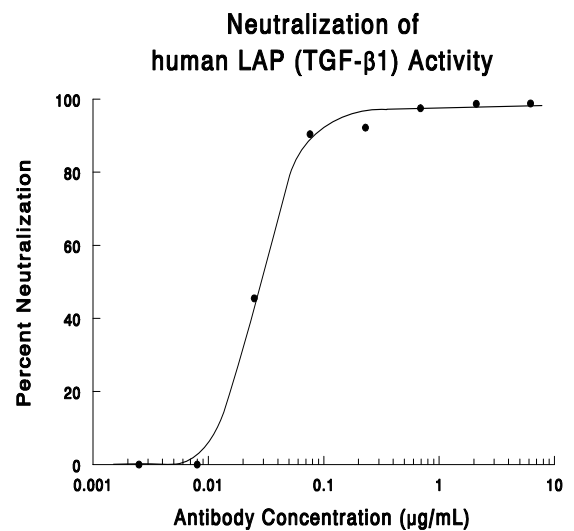


Figure 1

Human LAP (TGF-β1) inhibits TGF-β1 activity on a mouse T cell line, HT-2 (Tsang, M. *et al.*, 1995, Cytokine 7:389) in a dose-dependent manner. The ED₅₀ for this effect is typically 20 - 40 ng/mL in the presence of 0.25 ng/mL of TGF-β1.

Figure 2

To measure the neutralizing activity of antibody, various concentrations of LAP antibody were incubated with rhLAP (TGF-β1) for 1 hour at 22° C in a 96 well microtiter plate. Following this incubation, rhTGF-β1 was added to the plate, and the plate was incubated for another hour at 22° C. Following the second incubation period, HT-2 cells resuspended in rmlL-4 containing medium were added to the plate. The assay mixture, in a total volume of 100 μL, containing rhLAP at 125 ng/mL, rhTGF-β1 at 0.25 ng/mL, rmlL-4 at 7.5 ng/mL and LAP antibody at the concentrations indicated, was incubated for 48 hours at 37° C in a 5% CO₂ humidified incubator. Cells were pulsed with ³H-thymidine for the final 4 hours and then harvested onto glass fiber filters and the ³H-thymidine incorporated into DNA was determined. The ND₅₀ of the antibody is approximately 0.05 - 0.15 μg/mL.