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Product Information

Anti-Interleukin-12
produced in goat, IgG fraction of antiserum

Catalog Number **I7642**

Product Description

Anti-Interleukin-12 (IL-12) is developed in goat using recombinant mouse IL-12 expressed in the insect cell line *Sf* 21 as immunogen. The antibody is purified by Protein G affinity chromatography.

Anti-Interleukin-12 may be used in immunoblotting, ELISA, and neutralization. By ELISA, the antibody shows <10% cross-reactivity with recombinant human IL-12 and <5% cross-reactivity with recombinant porcine IL-12.

Interleukin-12 (IL-12) or Natural Killer Cell Stimulatory Factor (NKSF) is a disulfide-linked heterodimer of a 35 kDa light chain subunit and 40 kDa heavy chain subunit. The molecular mass of IL-12 is approximately 75 kDa. The p35 subunit of IL-12 shares amino acid sequence homology with IL-6 and G-CSF.[2] The p40 subunit has homology to the extracellular domain of the IL-6 receptor and to the ciliary neurotrophic growth factor receptor.[3,4] IL-12 is produced predominantly by monocytes and NK cells.[1] IL-12 induces T cells and NK cells to produce IFN-γ. Human IL-12 is not active on mouse cells, but mouse IL-12 is active on both mouse and human lymphocytes.[4]

Reagent

Lyophilized from 0.2 μm-filtered solution in phosphate buffered saline containing carbohydrates.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

To one vial of lyophilized powder, add 1 mL of 0.2 μm filtered phosphate buffered saline to produce a 1 mg/mL stock solution of the antibody. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

Storage/Stability

Prior to reconstitution, store at –20 °C. Reconstituted product may be stored at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at –20 °C. Avoid repeated freezing and thawing.

Procedure

Anti-Interleukin-12 is tested for its ability to neutralize the biological activity of recombinant mouse IL-12 on mouse splenocytes.[5] In this bioassay, 0.3 ng/mL recombinant mouse IL-12 is mixed with various dilutions of antibody in a 96-well plate for 1 hour at 37 °C. After preincubation, mouse splenocytes (2×10^5) and 10 ng/mL PMA are added to the antigen-antibody mixture. The assay mixture, in a total volume of 100 μL, is incubated at 37 °C for 48 hours in a humidified CO_2 incubator and pulsed during the final 4 hours with ^{3}H-thymidine. Cells are harvested onto glass filters and the ^{3}H-thymidine incorporation into DNA is measured.

The Neutralization $Dose_{50}$ (ND_{50}) of the antibody is defined as the concentration of antibody resulting in a one-half maximal inhibition of bioactivity of recombinant mouse IL-12 that is present at a concentration just high enough to elicit a maximum response.

Product Profile

ELISA: a working concentration of 0.5-1.0 μg/mL detects ~1 ng/well of recombinant mouse IL-12.

Immunoblotting: a working concentration of 1-2 μg/mL detects mouse IL-12. The detection limit for recombinant mouse IL-12 is ~20 ng/lane under non-reducing conditions. Under reducing conditions, the p40 and p35 bands from 20 ng/lane of recombinant mouse IL-12 can also be detected. Because this antibody preparation is a total IgG fraction, complete monospecificity cannot be assumed.

Endotoxin: <10 ng/vial by LAL method

References

1. Trinchieri, G., et al*., Progress in Growth Factor Research*, **4**, 355, (1992).
2. Merberg, D., et al., *Immunol. Today*, **13**, 77 (1992).
3. Gearing, D., et al., *Cell*, **66**, 9 (1991).
4. Schoenhaut, D., et al., *J. Immunol*., **148**, 3433 (1992).
5. Mattner, F., et al., *Eur. J. Immunol*., **23**, 2202 (1993).

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