Product No. I-3518
Lot 103H4857

Anti-Human Interleukin 3

Developed in Rabbit
IgG Fraction of Antiserum

Anti-Human Interleukin 3 (IL-3) is developed in rabbit using purified human recombinant IL-3 produced in *E. coli* as the immunogen. Whole antiserum is fractionated and then further purified by ion exchange chromatography to provide the IgG fraction of antiserum. This fraction is essentially free of other rabbit serum proteins. The product is supplied as a sterile-filtered liquid in 0.01 M phosphate buffered saline, pH 7.4, containing 0.1% sodium azide (see MSDS)* as a preservative.

Specificity

Rabbit Anti-Human IL-3 detects recombinant human IL-3 by dot blot immunoassay and radioimmunoassay. No reaction is observed versus recombinant mouse GM-CSF or recombinant human IL-2 by dot blot immunoassay and radioimmunoassay. No reaction is observed with recombinant human IL-4 and recombinant human IL-5 by dot blot immunoassay.

Description

Interleukin-3 is a multifunctional protein, originally called colony forming unit-stimulating activity (CFU-SA),[1] and is produced by activated T lymphocytes.[2] IL-3 supports the formation of multilineage colonies in the early development of multipotent hematopoietic progenitor cells. IL-3 induces colony formation of macrophages, neutrophils, mast cells and megakaryocytes from agar-suspended bone marrow cells.[3] IL-3 also interacts with IL-2 to stimulate growth of T lymphocytes[4] and to induce IgG secretion from activated B cells.[5] Other synonyms attributed to IL-3 include pan-specific hemopoietin, multicolony stimulating factor, mast cell growth factor, burst promoting activity, histamine-producing cell stimulating factor, P cell stimulating factor, and WEHI-3 factor.[6]

Uses

Rabbit Anti-Human IL-3 may be used to study human IL-3 using immunoblotting, dot blot, RIA, ELISA, or selective neutralization of human IL-3 bioactivity in cell culture.

Bioactivity

One ml of Rabbit Anti-Human IL-3 neutralizes a minimum of 170,000 Reference Units of recombinant human IL-3. Neutralization of proliferative activity is tested in culture using TF-1 cells.[7,8] One unit is defined as the amount of IL-3 required to induce a half-maximal incorporation of ^{3}H-thymidine. Activity is expressed in Reference units (NIBSC reference preparation for IL-3 code 88/780).

Dot Blot

A 1:4,000 dilution was determined using 100 ng recombinant human IL-3/dot on nitrocellulose membranes.

Protein Concentration: 8.5 mg/ml by extinction. $E_{280}^{1\%} = 14.0$.

RIA SYSTEM

RIA Characterization

The antiserum is characterized utilizing a second antibody-polyethylene glycol (PEG) RIA protocol, where 0.1 ml of a 1:20,000 dilution of antiserum has been found to bind at least 40% of 70 picograms of iodinated IL-3.

It is recommended that the antiserum be evaluated in the particular assay system chosen due to differences in systems and procedures.

RIA Reagents

1. Standards: Prepare and freeze aliquots of a stock standard solution of 10 ng/ml human IL-3 (Sigma Product No. I-7389) in dilution buffer. Thaw one aliquot for each assay and dilute in dilution buffer to the following concentrations: 10, 2.5, 0.63, 0.16, 0.04 ng/ml.
2. Dilution buffer: 0.01 M phosphate buffered saline, pH 7.8 containing 0.5% BSA and 0.1% sodium azide.

RIA Reagents (cont.)

3. Normal rabbit serum (Sigma Product No. R-9133): 2% in dilution buffer without BSA.
4. EDTA solution: Ethylenediaminetetraacetic acid (EDTA) disodium salt (Sigma Stock No. ED2SS): 0.1 M, pH 7.8 in distilled water. Adjust pH with 10 N NaOH.
5. Second antibody: Goat Anti-Rabbit IgG (Sigma Product No. R-0881) reconstituted in dilution buffer. Dilute reconstituted antiserum 1:5 in dilution buffer for use.
6. PEG solution: 6% PEG (Sigma Product No. P-2139, approximate molecular weight 8,000) in dilution buffer without BSA.

RIA Protocol

1. In polypropylene test tubes, add 0.2 ml sample or standard and 0.1 ml diluted antiserum.
2. Vortex the tubes.
3. Incubate for 1 hour at 37°C.
4. Add 0.1 ml I^{125} radioactive tracer diluted in dilution buffer.
5. Vortex the tubes.
6. Incubate for 2 hours at 37°C followed by an incubation of 18-20 hours at 4°C.
7. Add 0.1 ml EDTA solution and 0.1 ml 2% rabbit serum to all tubes.
8. Vortex the tubes.
9. Add 0.1 ml second antibody to all tubes.
10. Add 0.7 ml PEG solution to all tubes.
11. Vortex the tubes.
12. Incubate for 5 minutes at room temperature.
13. Centrifuge at 2000 x g for 15 minutes at 4°C.
14. Remove supernatant from each tube and determine the amount of radioactivity present in the precipitate.

RIA Sensitivity

Sensitivity is defined as the 90% intercept of a B/B_0 standard curve. In the above system the sensitivity has been found to be 15 pg/tube.

RIA Specificity

Specificity of the antiserum is defined as the ratio of antigen concentration to cross-reactant concentration at 50% inhibition of maximum binding. The cross-reactivity data obtained in the second antibody-PEG I^{125} RIA system is as follows:

Cross-Reactant	%Cross-Reactivity
Human IL-3, recombinant	100
Mouse GM-CSF, recombinant	<0.1
Human IL-2, recombinant	<0.1

(Dilutions for standard curve: 0.15 - 20.0 ng/ml; dilutions for cross-reactants: 25 - 100 ng/ml)

RIA Affinity Constant

The affinity constant (K_a) is determined by a Scatchard plot using this RIA system.
$K_a = 1.0 \times 10^{10}$ L/mole.

Dilution and Use

The contents of the vial may be further diluted in tissue culture media containing 10% serum or buffered saline containing 1% BSA, according to the planned application. If aseptic technique is used, additional filtration should not be necessary and should be avoided due to possible adsorption onto the filter membrane.

Storage

Store undiluted antibody at −20°C. The product should be stored frozen in working aliquots. Repeated freezing and thawing is **not** recommended. Storage in "frost-free" freezers is **not** recommended.

References

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