Product Information

Anti-Interleukin 6

Produced in Rabbit, IgG Fraction of Antiserum

I2143

Product Description

Anti-Interleukin 6 (IL-6) is produced in rabbit using purified human recombinant IL-6 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.

Interleukin-6 is a multifunctional protein originally discovered in the media of cells stimulated with double stranded RNA.[1] IL-6 appears to be directly involved in the responses that occur after infection and injury and may prove to be as important as IL-1 and TNF-α in regulating the acute phase response.[2,3] IL-6 is reported to be produced by fibroblasts, activated T cells, activated monocytes or macrophages, and endothelial cells. It acts upon a variety of cells, including fibroblasts, myeloid progenitor cells, T cells, B cells, and hepatocytes. IL-6 induces multiple effects, as indicated by its numerous synonyms: plasmacytoma growth factor (PCT-GF), interferon-β_2, monocyte derived human B cell growth factor, B cell stimulating factor (BSF-2), hepatocyte stimulating factor (HSF), and interleukin hybridoma/plasmacytoma-1 (IL-HP1). IL-6 also interacts with IL-2 in the proliferation of T lymphocytes.[5] IL-6 also potentiates the proliferative effect of IL-3 on multipotential hematopoietic progenitors.

Anti-IL-6 detects natural and recombinant human IL-6 by dot blot immunoassay. No reaction is observed against recombinant mouse IL-6, recombinant human IL-1β or recombinant human TNF-α.

Anti-IL-6 may be used to study human IL-6 using immunoblotting, dot blot, RIA, ELISA, or selective neutralization of human IL-6 bioactivity in cell culture.

Reagents

Supplied as 0.2 µm filtered solution in 0.01 M phosphate buffered saline, pH 7.4, containing 0.1% sodium azide as a preservative.

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.

Reconstitution

The contents of the vial may be further diluted with 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/Stability

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

Procedure

RIA System

RIA Characterization

Anti-IL-6 is characterized utilizing a second antibody-polyethylene glycol (PEG) RIA protocol, where 0.1 mL of a minimum 1:60,000 dilution of antiserum has been found to bind at least 40% of 40 picograms of iodinated IL-6.

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

RIA Reagents and Material Not Supplied

1. Standards: Prepare and freeze aliquots of a stock standard solution of 10 ng/mL human IL-6 (Cat. No. I7764) 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.
3. Normal rabbit serum (Cat. No. R9133) 2% in dilution buffer without BSA.
4. EDTA solution: Ethylenediaminetetraacetic acid (EDTA) disodium salt, (Cat. No. ED2SS) 0.1 M, pH 7.8 in distilled water. Adjust pH with 10 N NaOH.
5. Second antibody: Anti-Rabbit IgG (Cat. No. R0881) reconstituted in dilution buffer. Dilute reconstituted antiserum 1:5 in dilution buffer for use.
6. PEG solution: 6% PEG, (Cat. No. P2139), approximate molecular weight 8,000, in dilution buffer without BSA.
7. 2 mL Polypropylene test tubes.
8. 37 °C water bath for incubation.
9. Centrifuge.

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 ^{125}I 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 a minimum of 5 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 ^{125}I RIA system is as follows:

Cross-Reactant	% Cross-Reactivity
Human IL-6, recombinant	100
Mouse IL-6, recombinant	< 0.1
Human IL-1β, recombinant	< 0.1
Human TNFα, recombinant	< 0.1

RIA Affinity Constant

The affinity constant (K_a) is determined by a Scatchard plot using this RIA system.

K_a = at least 1.0 x 10^{10} L/mole.

Product Profile

One mL of Anti-IL-6 neutralizes a minimum of 900,000 Reference Units of recombinant human IL-6. Neutralization of proliferative activity is tested in culture using mouse T1165 cells.[5] One unit is defined as the amount of IL-6 required to induce a half-maximal incorporation of ^{3}H-thymidine. Activity is expressed in Reference units (NIBSC reference preparation for IL-6 code 88/514).

Protein concentration is determined by extinction, $E_{280}^{1\%}$ = 14.0.

References

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