Product Information Sheet for NR-3096

Polyclonal Anti-Human Interferon Gamma (antiserum, Rabbit)

Catalog No. NR-3096

This reagent is the property of the U.S. Government.

Lot (NIAID Catalog) No. G034-501-565

For research use only. Not for human use.

Contributor:

National Institutes of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH)

Product Description:

Reagent: Polyclonal antiserum to human interferon gamma
Host: Rabbit
Immunizing Antigen:
Human interferon gamma produced by human peripheral blood leukocytes stimulated with staphylococcal enterotoxin A and partially purified by control pore glass bead absorption and gel filtration
NIAID Class: Research Reference Reagent
Research Reference Reagent Note (attached): No. 34
Adjuvant used:
Equal volumes of Freund's complete and 30% Arlacel A in initial inoculations and Freund's incomplete in booster inoculations

Material Provided/Storage:

Composition: Lyophilized
Original Volume: 1.0 mL
Storage Temperature: 4°C or colder
Reconstitution: 1.0 mL sterile distilled water

Functional Activity:

Neutralizing Titer: 1:1300 against 10 Laboratory Units of human interferon gamma in Sindbis virus/human WISH cell microtiter assay
Antibody Cross-Reactivity-: No cross-reactivity against human interferon α or interferon β

Producer and Contract:

University of Texas Medical Brach, Galveston N01-AI-02659

Citation:

Acknowledgment for publications should read "The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: Polyclonal Anti-Human Interferon Gamma (antiserum, Rabbit), NR-3096."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2007; see www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

Disclaimers:

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1. Georgiades, J. A., M. P. Langford, G. J. Stanton, and H. M. Johnson. "Purification and Potentiation of Human Immune Interferon Activity." IRCS Medical Science 7 (1979): 559.
2. Langford, M. P., et al. "Large-Scale Production and Physiochemical Characterization of Human Immune Interferon." Infect. Immun. 26 (1979): 36–41. PubMed: 40881.

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3. Langford, M. P., et al. "Antibody to Staphylococcal Enterotoxin A-Induced Human Immune Interferon (IFN Gamma)." J. Immunol. 126 (1981): 1620–1623. PubMed: 6162895.
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5. Yamamoto, J. K., W. L. Farrar, and H. M. Johnson. "Interleukin 2 Regulation of Mitogen Induction of Immune Interferon (IFN Gamma) in Spleen Cells and Thymocytes." Cell. Immunol. 66 (1982): 333–341. PubMed: 6175428.
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RESEARCH REFERENCE REAGENT NOTE No. 34

Rabbit Antiserum to Human Gamma Interferon

Catalog Number G034-501-565

RESEARCH RESOURCES SECTION
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, Maryland 20025
August 1984

Preparation

Human gamma interferon (IFNγ) was prepared in roller bottle cultures of human peripheral blood leukocytes stimulated with staphylococcal enterotoxin A (SEA) and partially purified by a two-step procedure: control pore glass (CPG) bead absorption and elution with phosphate buffered saline containing 50% ethylene glycol and 1.0 M NaCl followed by Ultrogel AcA54 column purification (gel filtration) as previously described (1,2). Purified IFNγ preparations ($10^{4.8}$ to $10^{6.2}$ units/mg protein) containing approximately 10^5 units IFNγ were mixed with equal volumes of Freund's complete adjuvant and 30% Arlacel A and injected intramuscularly and/or subcutaneously on days 1, 4, 14, 43. In subsequent immunizations, IFNγ was mixed with equal volumes of Freund's incomplete adjuvant and injected subcutaneously and/or intramuscularly at monthly intervals as previously described for production of antibody to IFNγ (3).

For preparation of specific anti-globulin to human IFNγ, sera assayed to contain more than 100 units (1 unit antibody neutralizes 10 units of IFNγ) of neutralizing activity for IFNγ/ml were pooled. Pooled antisera were incubated at 56°C for 30 min and precipitated with 50% saturated ammonium sulfate by standard methods. After overnight refrigeration (4°C), the globulin precipitate was removed by centrifugation (3,000 x g for 20 min) and suspended in a volume of phosphate-buffered saline (PBS) equal to one-half the initial serum volume. After extensive dialysis against pH 7.2 PBS to remove ammonium sulfate, 10^7 human peripheral blood lymphocytes and 10^7 human amnion WISH cells were added to each ml of antiserum and incubated for 1 hr at 37°C. The cells were pelleted by centrifugation (1,000 x g for 10 min) and the serum was harvested. After repeating the cell absorption process with fresh cells, the anti-globulins were checked for the presence of contaminating antibodies to fetal calf serum, human albumin, human sera, mock IFNγ, and cell sonicates by the Ouchterlony immunoprecipitation method on microscope slides. Precipitation bands were observed against fetal calf serum (major contaminant), mock IFNγ, and cell sonicate. To remove these contaminating antibodies, 100 ml of Sepharose 4-B beads were activated with cyanogen bromide at pH 9.0 in the presence of 10 ml of fetal calf serum, 10 ml of concentrated mock IFNγ (10x), and 1 ml of cell sonicate. Excess and unbound proteins were eluted from the Sepharose beads by extensive washing with PBS. The beads were stored at 4°C for three days before use. Twenty-five ml of the antigen coupled Sepharose beads were placed in each 560 ml of antiglobulin to human IFNγ. After 1 h incubation with agitation, the beads were removed by centrifugation and 25 ml of fresh beads were added and the adsorption process repeated. Immunoadsorption effectively eliminated the antibodies to mock IFNγ, fresh leukocytes sonicate, and to fetal calf serum as determined by Ouchterlony immunoprecipitation from the antiglobulin to human IFNγ. No reduction in neutralizing activity to IFNγ was detected between precipitation and immunoadsorption. In addition, the antiglobulin to human IFNγ was tested for cross neutralization against human IFNα and IFNβ and no neutralizing

activity was detected (<30 units/ml). The pooled antiglobulins to IFNγ were aliquoted into vaccine vials (1.0 ml/vial), frozen at -70°C, lyophilized to dryness, and vacuum sealed.

Recommendations for Reconstitution

Add 1.0 ml of sterile distilled water or an appropriate medium to the lyophilized powder. The reconstituted globulin can be stored indefinitely at -20°C or lower.

Interferon Neutralization Assay

Half-log dilutions of test sera are mixed with equal volumes (0.2 ml) of IFNγ. The final concentration of IFNγ is approximately 10 units/ml. A unit of human IFNγ is defined as the concentration of IFN required to reduce Sindbis virus CPE by 50% on human WISH cells in microtiter plates. After 1 hr incubation at room temperature, the individual mixtures of antisera and IFN are added to target cells in triplicate (0.1 ml/well). Residual IFN titers are determined and the dilution of antiserum required to reduce 10 units IFNγ/ml to 1 unit is calculated. One unit of antiserum is the concentration expressed in 1 ml volume that will neutralize 10 units of IFNγ.

Potency

The interferon neutralizing titer of the 1.0 ml contained in the ampule was at least 1,800 units in our laboratory assay as of July, 1983 (1 unit neutralizes 10 units of IFNγ). Collaborative testing of the potency of the anti-globulin resulted in a mean titer of 1300 units/ml.

Results of Other Tests

The anti-globulin neutralizes the natural killing (NK) cell enhancing activity of human IFNγ (3-5), neutralizes the antiviral activity of human recombinant IFNγ, and neutralizes interleukin 2 enhancement of NK activity (5,6).

Use of Reference Antiserum

The purpose of this antiserum is to provide a reference reagent which can be used for the identification and characterization of biological and chemical properties attributed to human gamma interferon. The wide use of interferon in research has made it desirable to have standards which may be used to correlate data from different laboratories. This reagent is available in limited quantities and should be used only after preliminary studies have been performed.

The source of the reagent should be identified in each publication and a copy of all publications should be sent to the NIAID Antiviral Substances Program, National Institute of Allergy and

Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205.

Stability

Freeze-dried serum globulins are generally stable at room temperature (23°C) for indeterminant lengths of time. It is recommended, however, that the unopened ampules be stored at +4°C or lower temperatures. The reconstituted globulin should be stored at -20°C or lower.

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References

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2. Langford, M.P., Georgiades, J.A., Dianzani, F., Stanton, G.J., and Johnson, H.M. (1979) Infect. Immun. 26:36-41.

3. Langford, M.P., Weigent, D.A., Georgiades, J.A., Johnson, H.M., and Stanton, G.J. (1981) J. Immunol. 126:1620-1623.

4. Weigent, D.A., Langford, M.P., Fleischmann, W.R., and Stanton, G.J. (1982) In: Human Lymphokines (A. Khan and N.O. Hill, eds.), pp. 539-550, Academic Press, New York.

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