Product Information Sheet for NR-3094

Polyclonal Anti-Murine Interferon Gamma (antiserum, Rabbit)

Catalog No. NR-3094

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

Lot (NIAID Catalog) No. G032-501-565

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 murine interferon gamma
Host: Rabbit
Immunizing Antigen:
Mouse interferon gamma produced by stimulating murine spleen cells 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. 32
Adjuvant used:
Freund's complete plus 30% Arlacel A in initial inoculation 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: 1:800 against 10 Laboratory Units of murine interferon gamma
Antibody Cross-Reactivity-: No cross neutralization against other murine interferons

Producer and Contract:

University of Texas Medical Branch, 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-Murine Interferon Gamma (antiserum, Rabbit), NR-3094."

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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E-mail: contact@beiresources.org

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RESEARCH REFERENCE REAGENT NOTE No. 32

Rabbit Antiserum to Mouse Gamma Interferon

Catalog Number G032-501-565

RESEARCH RESOURCES SECTION
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, Maryland 20205
August 1984

Preparation

Mouse gamma interferon (IFNγ) was produced by stimulating mouse spleen cells with staphylococcal enterotoxin A (SEA) and partially purified by the two-step procedure used for purification of human IFNγ (CPG beads and Ultrogel AcA54) (1,2). Purified mouse IFNγ preparations ($10^{4.2}$ to $10^{5.8}$ units/mg protein), approximately 10^5 units, were emulsified with an equal volume of Freund's complete adjuvant and 30% Arlacel A and injected intramuscularly and/or subcutaneously on days 1, 4, 14, and 43. Then IFNγ preparations were mixed with Freund's incomplete adjuvant and injected intramuscularly or subcutaneously at monthly intervals as previously described for production of antibody to mouse IFNγ (3).

For preparation of specific anti-globulin to mouse IFNγ, sera were pooled which contained greater than 100 units of neutralizing activity/ml (1 unit neutralizes 10 units of IFNγ) to mouse IFNγ. The globulin (50% ammonium sulfate precipitate) fraction of the pooled sera was harvested and extensively dialyzed against PBS. The anti-globulin to mouse IFNγ as absorbed with fresh mouse spleen cells (10^7/ml anti-globulin), mouse L929 cells (10^7/ml anti-globulin) and twice with 25 ml of Sepharose beads that had fetal calf serum, mock IFNγ, and spleen cell sonicate proteins bound to them by the cyanogen bromide activation method. These immunoadsorption procedures eliminated gross contamination of antibodies to serum and cell proteins as detected by Ouchterlony immunoprecipitation on microscope slides. No significant reduction in neutralizing activity was detected after immunoadsorption and no cross neutralization was detected against other mouse IFNs (Newcastle disease virus induced IFN in mouse spleen cells and mouse L cell cultures). The pooled anti-globulins to mouse IFNγ were aliquoted into vaccine vials (1.0 ml), frozen, 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 mouse IFNγ is defined as the concentration of IFN required to reduce vesicular stomatitis virus (40-60 PFU/well) plaques by 50% on mouse L 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 820 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 800 units/ml.

Results of Other Tests

The anti-globulin neutralizes the natural killing (NK) cell enhancing activity of mouse IFNγ (4), neutralizes the antiviral activity of mouse recombinant IFNγ (5,6), neutralizes mitogen induced and recombinant IFNγ macrophage activating factor activity (7), and neutralizes interleukin 2 enhancement of NK activity (4,8).

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 mouse 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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