Product Information Sheet for NR-3095

Control for NR-3094 (antiserum, Rabbit)

Catalog No. NR-3095

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Lot (NIAID Catalog) No. G033-501-565

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: Control antiserum for NR-3094
Host: Rabbit
Immunizing Antigen: Culture supernatant fluids of unstimulated mouse spleen cells, partially purified by control pore glass bead absorption and gel filtration
NIAID Class: Research Reference Reagent
Research Reference Reagent Note (attached): No. 33
Adjuvant used: Freund's complete plus 30 % Arlacel A

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: No neutralizing activity against murine interferon gamma was observed

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: Control for NR-3094 (antiserum, Rabbit), NR-3095."

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.

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References:

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.
3. Osborne, L. C., J. A. Georgiades, and H. M. Johnson. "Classification of Interferons with Antibody to Immune Interferon." Cell. Immunol. 53 (1980): 65–70. PubMed: 6157486.

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RESEARCH REFERENCE REAGENT NOTE No. 33

Control Antiserum to Mouse Gamma Interferon

Catalog Number G033-501-565

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

Preparation

Mock* gamma interferon (IFNγ) was prepared from culture supernatants of unstimulated mouse spleen cells and partially purified by a two-step procedure: control pore glass (CPG) bead absorption and elution with phosphate-buffered saline (PBS), pH 7.2, and 1.0 M NaCl followed by Ultrogel AcA 54 column purification (gel filtration) as previously described (1,2). Mock IFNγ preparations 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 mock 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 human IFNγ (3).

For preparation of mock antiglobulin to mouse IFNγ, sera were pooled. The globulin (50% ammonium sulfate precipitate) fraction of the pooled sera was harvested and extensively dialyzed against PBS. The mock anti-globulin to human IFNγ was absorbed with 10^7 mouse spleen cells and 10^7 mouse L cells for each ml of mock antiserum by incubation for 1 hr at 37°C. The mock antiserum (560 ml) was then absorbed twice with 25 ml of Sepharose beads that had fetal calf serum, mock IFNγ, and mouse spleen cell sonicate proteins bound to it by the cyanogen bromide activation method. These absorption procedures eliminated gross contamination of antibodies to serum and cell proteins as detected by Ouchterlony immunoprecipitation on microscope slides. The pooled mock 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.

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*The initial agreement was to provide the reagents in two phases, half after the first year, and the balance after the second year. This was changed at the request of NIH at the end of the first year and the reagents were provided in one lot at the end of the second year. The constraints of the altered agreement resulted in insufficient mock antibodies produced as described above. Mock antibodies that were provided to NIH were thus processed normal rabbit sera that were compared to the above mock antibody preparations for neutralizing activity against IFNγ in both the mouse and human systems.

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

No neutralizing activity of mouse IFNγ was observed with the control antiserum.

Results of Other Tests

The control antiserum did not neutralize: the natural killing (NK) cell enhancing activity of mouse IFNγ, the antiviral activity of mouse recombinant IFNγ, and interleukin 2 enhancement of NK activity.

Use of Reference Antiserum

The purpose of this antiserum is to provide a reference reagent which can be used in conjunction with antiserum to mouse IFNγ for the identification and characterization of biological and chemical properties attributed to mouse 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 indeterminate 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

1. Georgiades, J.A., Langford, M.P., Stanton, G.J., and Johnson, H.M. (1979) IRCS Medical Science 7:559.

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

3. Osborne, L.C., Georgiades, J.A., and Johnson, H.M. (1980) Cell. Immunol. 53:65-70.