Product Information Sheet for NR-3087

Polyclonal Anti-Murine L-Cell Interferon (antiserum, Sheep)

Catalog No. NR-3087

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

Lot (NIAID Catalog) No. G024-501-568

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 α/β
Host: Suffolk-Hampshire female yearling sheep
Immunizing Antigen:
Mouse interferon prepared in L-929 cells induced by live Newcastle disease virus. Interferon contained approximately 20% murine interferon α and 80% murine interferon β. Booster inoculation was with L-cell interferon purified by affinity chromatography
NIAID Class: Research Reference Reagent
Research Reference Reagent Note (attached): No. 19
Adjuvant used: Freund's complete in booster inoculations

Material Provided/Storage:

Composition: Lyophilized
Original Volume: 0.5 mL
Storage Temperature: 4°C or colder
Reconstitution: 0.5 mL sterile distilled water

Functional Activity:

Neutralizing Titer: 1:300,000 against 8 to 10 Laboratory Units of mouse interferon (α and β)
Antibody Cross-Reactivity: Low levels of antibody to human leukocyte interferon

Purity:

Sterility: No evidence of bacterial or fungal contamination

Producer and Contract:

Medical College of Pennsylvania N01-AI-82568

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 L-Cell Interferon (antiserum, Sheep), NR-3087."

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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References:

1. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. "Protein Measurement with the Folin Phenol Reagent." J. Biol. Chem. 193 (1951): 265–275. PubMed: 14907713.
2. Armstrong, J. A. "Semi-Micro, Dye-Binding Assay for Rabbit Interferon." Appl. Microbiol. 21 (1971): 723–725. PubMed: 4325022.
3. Havell, E. A. and J. Vilcek. "Production of High-Titered Interferon in Cultures of Human Diploid Cells."

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Antimicrob. Agents Chemother. 2 (1972): 476–484. PubMed: 4670440.

4. Ogburn, C. A., K. Berg and K. Paucker. "Purification of Mouse Interferon by Affinity Chromatography on Anti-Interferon Globulin-Sepharose." J. Immunol. 111 (1973): 1206–1218. PubMed: 4728682.
5. Mogensen, K. E., L. Pyhala and K. Cantell. "Raising Antibodies to Human Leukocyte Interferon." Acta Pathol. Microbiol. Scand. [B] 83 (1975): 443–450. PubMed: 1180059.

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NR-3087

RESEARCH REFERENCE REAGENT NOTE #19

SHEEP ANTISERUM TO MOUSE L-CELL INTERFERON

CATALOG NUMBER G-024-501-568

Research Resources Branch
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, Maryland 20205

August, 198

Preparation

Antibodies to mouse L-cell interferon were produced in a Suffolk-Hampshire female yearling sheep based on the procedure of Mogensen, et al. (1). The sheep received twelve weekly injections of 1.3×10^7 units per injection of mouse interferon prepared in L-929 cells (2) induced by live Newcastle disease virus (3) and purified to a specific activity of 1×10^6 units per mg protein of bovine plasma albumin-sepharose (4). The interferon preparations contained approximately 20 percent MuIFN-α (22-26,000 daltons) and 80 percent MuIFN-β (36-40,000 daltons)* as determined by gel filtration on Sephadex G100. Six weeks after the twelfth injection, a booster inoculation of 2.5×10^7 units of L-cell interferon purified by antibody affinity chromatography (3) to a specific activity of 2.0×10^7 units per mg protein was admixed with Freund's Complete Adjuvant and injected into several intramuscular sites. Bleedings were begun seven days later. Other booster injections, followed by bleedings and six week rest periods were carried out until maximum antibody titers were achieved. Sera of maximum titer were used for preparing this reference standard.

Greater than 95 percent of the antibodies to known contaminants present in the interferon preparation used for immunization were removed by immunoabsorption techniques utilizing antigens bound to Sepharose 4B. The antigens were those components of an interferon preparation which did not bind to absorbed anti-interferon globulin bound to Sepharose. The globulin portion of the serum was separated by precipitation with 50 percent ammonium sulfate, dialyzed versus 0.01 M sodium phosphate buffer pH 7, then sterilized by filtration. The globulin was dispensed (0.5 ml per ampule), freeze-dried and sealed by the American Type Culture Collection.

Recommendations for Reconstitution

Add 0.5 ml of sterile physiologic saline solution or an appropriate medium to the lyophilized powder. Precautions should be taken to avoid loss of material in the neck or stem of the ampule. The reconstituted globulin can be diluted and stored indefinitely at -20°C or lower.

Interferon Neutralization Assay

The assay procedure used at The Medical College of Pennsylvania is similar to the interferon assay in microtiter plates (5, 6), except that 50 µl volumes of serial two-fold dilutions of antiserum are preincubated for 1 hr at 37°C with 50 µl of graded interferon dilutions covering the range from 1-32 units before addition of the 50,000 mouse L-929 cells per well. Encephalomyocarditis virus at a multiplicity of 0.2 was used for challenge. Interferon, virus and cell controls are included in each test. The antiserum is titrated against several dilutions of test antigens in order to select, for computation of the titer, the series with the appropriate number of interferon units available for neutralization by antibody. The highest dilution which neutralized 8 - 10 reference units of interferon by partially restoring viral cytopathic effect, corrected for 1 ml volume, represented the titer of the antiserum. The mouse interferon reference standard used was G-002-904-511.

* Terminology recommended by the Committee on Interferon Nomenclature, 1980.

Potency

The interferon neutralizing titer of the 0.5 ml contained in the ampule is 300,000 (versus 8-10 reference units of interferon). The antiserum titer is based on the combined results of assays performed in twelve laboratories, including our own. The figures received from all laboratories were adjusted to indicate a titer relative to 50 percent neutralization of 8-10 reference units (standard G-002-904-511) of mouse interferon. Refer to the interferon neutralization assay procedure.

The interferon neutralizing titer does not indicate the number of units of interferon an antiserum is able to neutralize. The actual number of units of interferon the contents of the ampule can neutralize is approximately 750,000. This figure is derived by: (a) correcting for the number of reference units used in the neutralization test; and (b) correcting the cytopathic effect from a 50 to 100 percent endpoint as shown below:

> An interferon neutralizing titer of 300,000 relative to 10 units of interferon is equivalent to a titer of 3,000,000 relative to one unit of interferon. Correcting the cytopathic effect from 50 to 100 percent in our procedure usually requires 2-two fold dilution steps. Therefore, using 4 as the projected correction factor, the number of units that can be neutralized is approximately 750,000 (i.e. 3,000,000 ÷ 4).

This hyperimmune antiserum can neutralize fully both MuIFN-α and MuIFN-β species of mouse interferon derived from L-929 cells.

This antiserum also has low levels of antibodies to human leukocyte interferon (7).

Results of Other Tests

Tests on randomly selected ampules showed no detectable bacterial or fungal growth. The 0.5 ml of globulin is equivalent to 15.5 mg protein by the Lowry procedure (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 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 strongly recommended, however, that the unopened ampules be stored at +4°C or lower temperatures. The reconstituted globulin can be kept at +4°C, but a temperature of -20°C or lower is advised for long term storage.

Reagent Control

A control globulin preparation for this antibody to mouse L-cell interferon is also available (G-025-501-568).

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References

1. Mogensen, K.E., Pyhälä, L. and Cantell, K. (1975) Acta Path. Microbiol. Scand., Sect. B 83:443-450.

2. Sanford, K.K., Earle, W.R. and Likely, G.D. (1948) J. Natl. Cancer Inst. 9:229-246.

3. Ogburn, C.A., Berg, K. and Paucker, K. (1973) J. Immunol. 111:1206-1218.

4. Davey, M.W., Sulkowski, E. and Carter, W.A. (1976) J. Virol. 17:439-445.

5. Havell, E.A. and Vilček, J. (1972) Antimicrob. Agents and Chemother. 2:476-484.

6. Armstrong, J.A. (1971) Appl. Microbiol. 21:723-725.

7. Paucker, K. and Dalton, B.J. (1980) Proc. N.Y. Acad. Sci., Conference on Regulatory Functions of Interferon. In Press. 350:332-338

8. Lowry, O.H., Rosebrough, J., Farr, L. and Randall, R.J. (1951) J. Biol. Chem. 193:265-275.