Product Information Sheet for NR-3090

Control for NR-3089 (antiserum, Sheep)

Catalog No. NR-3090

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

Lot (NIAID Catalog) No. G027-501-568

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: Control antiserum for NR-3089
Host: Suffolk-Hampshire female yearling sheep
Immunizing Antigen: Components of crude human leukocyte interferon alpha preparations that did not bind to anti-human leukocyte interferon alpha affinity column (i.e. void volume)
NIAID Class: Research Reference Reagent
Research Reference Reagent Note (attached): No. 23
Adjuvant used: Freund's complete adjuvant in initial and 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: < 1:50 against 8 to 10 Laboratory Units of human leukocyte interferon alpha

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: Control for NR-3089 (antiserum, Sheep), NR-3090."

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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NR - 3090

RESEARCH REFERENCE REAGENT NOTE # 23

CONTROL ANTISERUM (SHEEP) TO HUMAN LEUKOCYTE INTERFERON

CATALOG NUMBER G-027-501-568

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

March, 1981

RESEARCH REFERENCE REAGENT NOTE #_____
CONTROL ANTISERUM (SHEEP) TO HUMAN LEUKOCYTE INTERFERON
CATALOG NUMBER G-027-501-568

Preparation

This serum globulin was prepared as a correlate to Sheep Antiserum to Human Leukocyte Interferon (G-026-502-568). The materials used for immunization (hereafter referred to as void) were those components of crude human leukocyte interferon (CIF) preparations (1) which do not bind to absorbed anti-interferon globulin (2) bound to Sepharose 4B. This material is routinely recovered during the preparation of partially purified human leukocyte interferon by affinity chromatography. Materials selected for immunization showed greater than 99% reduction in antiviral activity. The rationale for using void is as follows: in addition to interferon, induced cells probably release into the growth medium numerous other cellular proteins. Selective affinity chromatography ideally retains interferon but does not remove in substantial amounts other induced or normal cell products. Immunization with this material should, therefore, yield an antiserum containing antibodies to all such products. Since the innoculum was not completely devoid of interferon, low levels of antibody to human leukocyte interferon may be present in this antiserum.

A female Suffolk yearling sheep received three intramuscular injections of 50 mg void. The first two innoculations contained Freund's Complete Adjuvant. Antiserum was collected for a five week period starting seven days after the third injection. After a six week rest, a booster injection of void with adjuvant was given, and the bleeding cycle was repeated seven days later. This pattern was followed until adequate quantities of serum were collected. 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 (3,4), except that 50 µl volumes of serial two-fold dilutions of antiserum were preincubated for 1 hour at 37°C with 50 µl of graded interferon dilutions covering the range from 1-32 units before addition of the 30,000 human FS-4 cells per well. Encephalomyocarditis virus at a multiplicity of 0.2 was used for challenge. Interferon, virus and cell controls were included in each test. The antiserum was titrated against several dilutions of test antigen in order to select, for computation of the titer, the series with the appropritae 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 human leukocyte interferon reference standard used was G-023-901-527.

Passive Hemagglutination Test

Antibody titers to three known contaminants found in the interferon preparation were measured by passive hemagglutination using the procedure of Boyden (5) as modified by Ogburn, et al. (6). Washed sheep red blood cells were treated with tannic acid (25 µg/ml) and incubated with the test antigens for 30 minutes at 37°C to facilitate binding of antigens to the surface of the red blood cells. The cells were then washed to remove unbound antigen and used as the indicator in the assay. Serial dilutions of the heat inactivated antiserum were made in a round bottom microtiter plate (Linbro Scientific #76-311-05), and 0.05 ml of a 0.6% solution of the antigen-coated sheep red blood cells was added to each well. Controls included known positive sera and antigen coated sheep red blood cells in diluent alone (PBS containing 1% normal rabbit serum). The mixture was incubated at room temperature for four hours and the pattern of hemagglutination was recorded. The presence of antibodies to the test antigen is indicated by agglutination of the antigen-coated red blood cells. The titer of the serum is defined as the reciprocal of the highest dilution showing complete hemagglutination, corrected for a one milliliter volume.

Potency

The anti-interferon titer is based on the combined results of assays performed in ten laboratories. The titers received from all laboratories were corrected to indicate a titer per milliliter for the 50 percent neutralization of 8-10 reference units of human interferon. The assigned titer of this control serum is negligible (less than 50 interferon neutralizing units per 0.5 ml).

Presumably, this antiserum contains antibody to numerous antigens of cell, virus and medium origin. Development of antibodies to three antigens known to be present (egg albumin, human albumin and an extract of human leukocyte) were monitored by passive hemagglutination throughout the immunization procedure. Antibody titers to these antigens were very similar to those found in unabsorbed antisera of a sheep immunized with partially purified human leukocyte interferon (G-026-502-568).

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 17 mg protein by the Lowry procedure (7).

Use of Reference Antiserum

The purpose of this antiserum is to provide a reference reagent which can be used in conjunction with Sheep Antiserum Human Leukocyte Interferon (G-026-502-568) for the identification and characterization of biological and chemical properties attributed to human leukocyte 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 & Infectious Diseases, National Institues of Health, Bethesda, Maryland 20205.

Stability

Freeze-dried serum globulins are generally stable at room temperature (25°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.

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