Product Information Sheet for NR-3078

Human Leukocyte Interferon Alpha

Catalog No. NR-3078

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Lot (NIAID Catalog) No. Ga23-902-530

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: Human Leukocyte Interferon Alpha
NIAID Class: WHO International Standard
Research Reference Reagent Note (attached): No. 29
Titer: 12,000 International Units/ampoule
Molecular Weight: 15,000 daltons and 20,000 daltons

Method of Preparation:

Tissue Culture System: Human leukocytes induced with Sendai virus
Medium: Tricine-buffered Minimum Essential Medium + 5% human serum
Treatment: Purified by Cantell's method of differential precipitation to obtain fraction PIF-A. Suspended in 0.1 M sodium phosphate buffer, pH 7 supplemented with human serum albumin (5 mg/mL)
Freeze-drying: Residual moisture 3%; back-filled with argon and heat-sealed at atmospheric pressure

Material Provided/Storage:

Composition: Freeze-dried
Original Volume: 1.0 mL
Storage Temperature: -70°C or colder
Reconstitution: 1 mL sterile distilled water
Stability after Freeze-Drying: No loss of activity during heating from 50°C to 90°C over a 28 hour period. Product is estimated to have unlimited stability at -20°C and -70°C

Purity:

Activity on Heterologous Cells:
5.5×10^4 Laboratory Units/mL in human A549 cells
3.7×10^4 Laboratory Units/mL in bovine ERTr cells
6.2×10^4 Laboratory Units/mL in feline FEA cells
4.2×10^2 Laboratory Units/mL in murine L cells
Sterility: No evidence of mycoplasmal, bacterial or fungal contamination

Producer and Contract:

Medical College of Wisconsin N01-AI-02658

Citation:

Acknowledgment for publications should read "The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: Human Leukocyte Interferon Alpha, NR-3078."

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. Kauppinen, H. L., G. Myllyla, and K. Cantell. "Large Scale Production and Properties of Human Leukocyte

Interferon Used in Clinical Trails." Adv. Exp. Med. Biol. 110 (1978): 1–13. PubMed: 215010.

2. Jameson, P., D. Greiff, and S. E. Grossberg. "Thermal Stability of Freeze-Dried Mammalian Interferons. Analysis of Freeze-Drying Conditions and Accelerated Storage Tests for Murine Interferon." Cryobiology 16 (1979): 301–314. PubMed: 226331.
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4. Cantell, K., S. Hirvonen, and V. Koistinen. "Partial Purification of Human Leukocyte Interferon on a Large Scale." Methods Enzymol. 78 (1981): 499–505. PubMed: 6173635.
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NR-3078

RESEARCH REFERENCE REAGENT NOTE No. 29

Freeze-dried Reference Human Interferon Alpha [HuIFN-α (Leukocyte/Sendai)]
Catalog Number Ga23-902-530

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

Freeze-dried Human Interferon Alpha (Leukocyte/Sendai) Reference (Ga23-902-530)

Preparation: Human interferon alpha [HuIFN-α(Leukocyte/Sendai)] was prepared by Dr. Kari Cantell (Helsinki, Finland) by Sendai virus infection of human leukocytes suspended in tricine-buffered minimal essential medium supplemented with 5% human serum (1). After one day of incubation, the supernatant fluids were collected following centrifugation, and the IFN was purified by Dr. Cantell using his method of differential precipitation to obtain fractions P-IF A and P-IF B (2). Equal parts of these two fractions were mixed and sent to the Medical College of Wisconsin (MCW). The mixture, having a specific activity of 3.6×10^6 IU/mg, was stored at -70°C. Subsequently, the sterile IFN preparation was aseptically diluted into ice-cold, sterile buffer solution composed of 0.1 M sodium phosphate buffer, pH 7, supplemented with 5 mg/ml human serum albumin (Travenol "Buminate"). The vessel was packed in wet ice to keep the solution chilled during the process of filling the ampoules; 1.00-ml portions were dispensed into borosilicate glass ampoules using a high-precision Hamilton dispenser. The reproducibility of the fill, as measured by the weight of liquid dispensed into 24 preweighed vials (distributed throughout the fill), was 0.12% (coefficient of variation). Ampoules were filled in groups of 24, and held on ice until 5 groups were filled and were then placed in the refrigerated chamber of the freeze-dryer. After all ampoules were filled, they were frozen at -30°C, and the material was dried to a residual moisture of about 3%. The ampoules were then backfilled with argon and heat-sealed at atmospheric pressure. The last ampoule filled in each group of 24 was marked for testing of sterility and antiviral activity after freeze-drying. Ampoules are stored at -70°C but can be shipped at ambient temperatures.

Recommendations for reconstitution: 1.0 ml of sterile distilled water should be added to the lyophilized powder, with care being taken to avoid loss of any material in the neck or stem of the ampoule. Small portions of the reconstituted IFN may be stored at -70°C for dilution at another time. However, a suitable amount of an appropriate dilution, based on the known sensitivity of the assay being used, should be made in the freeze-drying buffer (see above) supplemented with HSA, 5 mg/ml, or in serum-containing culture medium used in the biological assay. Aliquots of the diluted IFN should preferably be stored at -70°C in volumes each sufficient for a single titration. It may be possible to store enough material in a single container at -70°C for use in as many as 3 titrations; more extensive repeated thawing and freezing can result in loss of activity. All liquid samples should be stored at -70°C or lower.

Stability: The freeze-dried reference preparation did not lose any activity in the linear non-isothermal accelerated degradation test (3) in which material is progressively heated from 50°C to 90°C over a 28-hour period. From the results of the predictive multiple isothermal accelerated degradation test (3), involving storage at 52°C, 60°C, 68°C, and 76°C for periods up to 1 year, the product is estimated to have unlimited stability at -20°C and -70°C. The time predicted to lose 1 log of activity at temperatures above freezing was estimated from these data to be 0.38 years at 56°C, 2.8 years at 37°C, 18.1 years at 20°C, and 144 years at 4°C.

Test results: No mycoplasma, bacteria or fungi were detected in 94 samples tested from the 113 different groups of ampoules composing the reference lot. The preparation contains no detectable viruses, as indicated by lack of cytopathic effect during blind passage in cultures of human heteroploid A549 cells or mouse L cells, and by lack of interference with EMCV replication in the same cell cultures. The IFN used for freeze-drying was diluted to contain 1 mg HSA/ml and characterized as follows: it was non-sedimentable at 100,000 x g, more than 99% inactivated by trypsin in 1 hr, stable during heating at 56°C for up to 3 hrs, and 38% inactivated during 6 hrs of pH 2 dialysis at 4°C, without furter loss of activity through a total of 48 hrs at pH 2. The product was not neutralized by antisera to HuIFN-γ (either provided by Irwin Braude, Meloy Labs, Springfield, VA, or prepared at MCW against purified HuIFN-γ), or by anti-IFN-β serum (NIH G028-501-568); but it was neutralized completely by anti-HuIFN-α serum (NIH G026-502-568). The IFN was composed of two molecular sizes of 15,000 daltons and 20,000 daltons, as estimated by sodium-dodecyl-sulfate polyacrylamide-gel electrophoresis in phosphate buffer by the method of Weber and Osborne. Analysis of HuIFN-α by isoelectric focusing revealed two approximately equal peaks of activity with isoelectric points of 5.6 and 6.1.

Potency was determined from the data contributed by eight laboratories which had performed two or more titrations of the preparation using a microtiter modification of a proposed reference bioassay technique (Table 1) (4,5). The reference bioassay involves the reduction in yield of infectious EMCV in the A549 line of human lung carcinoma cells; EMCV yields were measured in L cells. The geometric mean titer (GMT) calculated as the mean of the GMT values reported from each laboratory (total number of titrations = 44) was 4.28 log units/ml/ampoule (with a standard deviation, S.D., of 0.47 log, corresponding to about 3.0-fold variation). Titration of the HuIFN-α (Leukocyte/Sendai) by routinely used bioassays of different types with various cell-virus combinations, mostly dye-uptake measurements of cytolysis, gave GMT values ranging from 3.08 to 4.29 log units/ml, with a mean of 3.66 log units/ml (S.D. 0.49). Additional information is provided in Table 1. There was considerable activity on cells of heterologous species, characteristic of this type of IFN, with the following observed unadjusted titers obtained by the EMCV hemagglutination yield-reduction method (6): 5.5×10^4 Laboratory Units (LU)/ml in human A549 cells, 3.7×10^4 LU/ml in bovine EBTr cells, 6.2×10^4 LU/ml in feline FEA cells, and 420 LU/ml in murine L cells.

Titer assignment: The assigned titer of the HuIFN-α (Leukocyte/Sendai) NIH Reference Reagent Ga23-902-530 is derived from the test results of an international collaborative study using a proposed reference bioassay by proportional relationship to the International Reference Preparation, Human Leukocyte Interferon, British MRC 69/19 having an assigned potency of 5,000 IU (see Table 1). The assigned potency of Ga23-902-530 is 12,000 or 4.08 $\log_{10}$ International Units (IU)/ampoule.

Use of Reference Interferon: The purpose of the HuIFN-α (Leukocyte/Sendai) Reference Interferon Reagent is to provide a comparison of the sensitivities of bioassays that measure the antiviral activity of HuIFN-α (Leukocyte/Sendai) in different laboratory preparations of HuIFN-α (Leukocyte/Sendai) which have dose response curves parallel to that of the Reference Reagent (4,5,7-9). It

should be noted that if the number or proportion of different IFN-α subtypes in a given leukocyte IFN preparation under test is known to differ significantly from that in this reference preparation, then the use of this reference preparation may not be appropriate. Each laboratory should measure the HuIFN-α (Leukocyte/Sendai) Reference Reagent simultaneously with an internal laboratory standard in five or more titrations done on separate occasions, and should report the observed logarithm of the geometric mean titer and its standard deviation along with the assigned titer (as the logarithm) of the Reference Reagent Interferon according to recommendations by the World Health Organization (4,5,7-9). The number of International Units (IU)/ml in the laboratory standard (lab std.) should be calculated by proportional relationship to the Reference Reagent (Ref. IFN) as follows:

$$(1) \quad \frac{\text{NIH Ref. IFN assigned IU}}{\text{GMT of NIH Ref. IFN observed LU}} \times \text{GMT lab std. observed LU} = \text{lab std. IU}$$

Similarly, the laboratory standard may be used to determine the titer of test samples in IU.

$$(2) \quad \frac{\text{lab std. IU [from (1)]}}{\text{GMT of lab std. observed LU}} \times \text{GMT test sample observed LU} = \text{test sample IU}$$

References:

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Table 1. Summary of results of the international collaborative study of the Human Interferon Alpha (Leukocyte/Sendai) Reference Preparation NIH catalog number Ga23-902-530

Assay method	#1	#2	#3	#4	#5	#6	#7	#8	Summary per total labs[b]	Summary per total tests[c]
EMCV yield-reduction[a]										
Number of titers	10	5	2	6	8	5	5	3	8	44
GMT (log)	5.11	4.52	4.54	4.35	4.02	4.27	3.75	3.68	4.28*	4.37*
S.D. (log)	0.36	0.15	0.18	0.15	0.13	0.75	0.46	0.82	0.47	0.61
Other assay methods										
Number of titrations	24	5	NT	NT	7	6	5	7	6	
GMT (log)	3.27	4.26	-	-	4.22	4.71	3.26	3.84	3.93	
S.D. (log)	0.13	0.09	-	-	0.29	0.18	NA	0.12	0.58	

a/ The reference bioassay method measured the reduction in yield of encephalomyocarditis virus (EMCV) in the human A549 cell line; yield of infectious EMCV was measured by titrations in L cells using a cytopathic-effect endpoint. A standard protocol modified from that originally recommended (4,9) detailing the steps in the microtiter method was provided all participants. EMCV and both cell lines were also provided by Dr. Grossberg's laboratory at the Medical College of Wisconsin.

b/ In this column the geometric mean titer (GMT) and standard deviation (S.D.) are based on the GMT values obtained from titers calculated from the raw data provided by each laboratory. An S.D. value calculated so as to provide a combined estimate of random variation between titrations within the individual laboratory is 0.40 log corresponding to about 2.5-fold variation.

c/ In this column the GMT and S.D. are based on the total number of titers obtained without regard to laboratory.

* The assigned potency of Ga23-902-530, in relation to the International Reference Preparation of Human Leukocyte Interferon 69/19, is 12,000 or 4.08 $\log_{10}$ International Units/ampoule (see text).