Product Information Sheet for NR-3083

Human Recombinant Interferon Alpha 2a (Alpha A) (rHuIFN-α2a)

Catalog No. NR-3083

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

Lot (NIAID Catalog) No. Gxa01-901-535

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 Recombinant Interferon Alpha 2a (Alpha A) (rHuIFN-α2a)
NIAID Class: WHO International Standard
Research Reference Reagent Note (attached): No. 31
Titer: 9,000 International Units/ampoule
Molecular Weight: 18,500 daltons

Method of Preparation:

Producer System: *E coli* cells transformed with a plasmid derived from pLeIFA25
Medium: Nutrient medium
Treatment: Purified by serial chromatography. Suspended in sodium chloride (9 mg/mL) with human serum albumin (5 mg/mL) pH 6.9
Freeze-drying: Lyophilized and sealed under nitrogen

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 (expressed as % of activity observed in human WISH cells):
Monkey, Vero (9%)
Bovine, MDBK (150%)
Guinea Pig transformed (30%)
Feline, Felung (130%)
Cell lines of the following species: mouse, rat, rabbit, hamster and horse (< 1%)
Sterility: No evidence of microbial growth

Producer and Contract:

Hoffman LaRoche, Inc. Nutley, New Jersey

Citation:

Acknowledgment for publications should read "The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: Human Recombinant Interferon Alpha 2a (Alpha A) (rHuIFN-α2a), NR-3083."

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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NR-3083

RESEARCH REFERENCE REAGENT NOTE No. 31

Freeze-dried Reference Human Recombinant Alpha 2 Interferon
Catalog Number Gxa01-901-535

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

Freeze-dried Human Interferon Alpha 2

Preparation: Recombinant Human Alpha 2 Interferon (HuIFN-α2), also designated as recombinant IFN-αA, was prepared at the Roche Research Center in Nutley, New Jersey. E. coli cells transformed with a plasmid derived from pLeIFA25 (1) were grown in nutrient medium under conditions that permitted the organism to produce HuIFN-α2. The cells containing HuIFN-α2 were killed by treatment at low pH (ca. 1.8), harvested, passed through a mechanical grinder and suspended in extraction buffer. The resultant cell debris and nucleic acids were flocculated and removed by centrifugation. The HuIFN-α2 in the supernatant fluid was purified by a series of procedures involving affinity chromatography on immobilized anti-interferon monoclonal antibodies, cation exchange chromatography (2), and molecular exclusion chromatography.

The highly purified HuIFN-α2 was diluted to the required concentration with a solution containing sodium chloride (9 mg/ml) and human serum albumin (5 mg/ml). The pH was adjusted to 6.9 with sodium hydroxide and the solution was filter sterilized. One ml aliquots were aseptically dispensed into sterile ampoules. The contents of the ampoules were lyophilized and the ampoules sealed under nitrogen. The reproducibility of the fill, as measured by determination of the protein content of 20 ampoules, was + 8.8% (coefficient of variation).

Recommendations for reconstitution: 1.0 ml of sterile distilled water should be added to the lyophilized powder, care being taken to avoid loss of any material in the neck or stem of the ampoule. Portions of this reconstituted material can be stored at -70°C, or an appropriate (e.g., 1:10) dilution can be made, preferably in 0.1 M sodium phosphate buffer pH 7 containing 5 mg/ml human serum albumin (HSA); Hanks' salt solution with 5 mg/ml HSA or serum-containing culture medium may be substituted. For optimum, long-term preservation of stability, storage of samples of the liquid material should be at -70°C.

Stability: The freeze-dried reference preparation did not lose any activity in the linear non-iothermal 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 2.46 years at 56°C, 16 years at 37°C, 94.5 years at 20°C, and 679 years at 4°C.

Test results: Forty containers were tested for sterility according to the USP Direct Plant Method. The vials were reconstituted with sterile water and 20 vials were tested in fluid thioglycollate medium and 20 in trypticase soy broth. No evidence of microbial growth was observed during the incubation period.

The amino acid sequence of the HuIFN-α2 used to prepare this standard differs from that predicted from the DNA sequence reported by Streuli et al. (5) in that the amino acid at position 23 is lysine instead of arginine. The purity

was 99% as determined by photometric scanning of gels following non-reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Its molecular weight was estimated to be 18,500. It was non-sedimentable at 100,000xg for 90 minutes, stable at pH 2, and inactivated by trypsin. The specific activity of the HuIFN-α used was 2×10^8 International Units/mg protein as determined in a cytopathic effect-reduction assay (5) with WISH cells and vesicular stomatitis virus (VSV) as challenge.

Potency was determined from data contributed by eight international laboratories which had performed two or more titrations of the preparation using a microtiter modification of a proposed reference bioassay technique (Table 1) (6,7). 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=43) was 4.13 log units/ml/ampoule (with a standard deviation (S.D.) of 0.31 log corresponding to about 2.0-fold variation). Titration of the HuIFN-α2 by routinely used bioassays of different types with various virus-cell combinations, mostly dye-uptake measurements of cytolysis, gave GMT values ranging from 2.92 to 4.46 log units/ml, with a mean of 3.71 log units/ml and S.D. of 0.59. Greater detail is provided in Table 1 and its footnotes. Antiviral activity expressed as a % of that observed in human WISH ammion cells was observed in the following cell lines: monkey, Vero 9%; bovine, MDBK 150%; guinea pig transformed (30%); and feline Felung 130%. Negligible activity (<1% of that in human WISH cells) was observed on cell lines of the following species: mouse, rat, rabbit, hamster, and horse.

Titer assignment: From the test results of an international collaborative study using a reference bioassay, the assigned titer of the HuIFN-α2 Reference Preparation Gxa01-901-535 is 9000 International Units/ampoule or 3.95 $\log_{10}$ International Units/ampoule.

Use of reference interferon: The purpose of the HuIFN-α2 Reference Preparation is to provide a comparison of the sensitivities of bioassays that measure the antiviral activity of HuIFN-α2 in different laboratories. Each laboratory should measure the HuIFN-α2 Reference Preparation in comparison with its own HuIFN-α2 internal laboratory standard preparation. The WHO recommends that five or more titrations of the reference and the laboratory standard preparation should be done, and that the observed logarithm of the geometric mean titer and its standard deviation be reported in each publication along with the assigned titer (as the logarithm) of the Reference Interferon Standard (6-10). A large number of aliquots of the calibrated laboratory standard material should be kept frozen at -70°C and titrated every time an assay of a HuIFN-α2 sample is run. WHO further recommends that the titers of all samples measured in interferon bioassays be reported in International Units provided that the correction of observed titers be made on the basis of proportional relationships among the preparations, as illustrated below (where IU = International Units, and LU = Laboratory Units):

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

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

To be able to do this, dose-response curves of these materials must be parallel (6-10).

References:

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Table 1. Summary of results of the international collaborative study of the Human Recombinant Interferon Alpha 2 Reference Preparation NIH catalog number Gxa01-901-535

Assay method	Results obtained per laboratory								Summary per total	
	#1	#2	#3	#4	#5	#6	#7	#8	labs[b/]	tests[c/]
EMCV yield-reduction[a/]										
Number of titers	10	5	2	5	8	5	5	3	8	43
GMT (log)	4.60	4.44	4.22	4.13	4.00	3.90	3.59	4.19	4.13*	4.17*
S.D. (log)	0.44	0.21	0.48	0.31	0.49	0.97	0.34	0.53	0.31	0.57
Other assay methods										
Number of titrations	24	5	NT	NT	7	6	5	7	6	
GMT (log)	2.92	4.11			4.04	4.46	3.23	3.52	3.71	
S.D. (log)	0.18	0.11			0.30	0.25	NA	0.19	0.59	

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 recc mended (6,7) 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 0.51 log corresponding to about 3-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 Gxa01-901-535, in relation to the International Reference Preparation of Human Leukocyte Interferon 69/19, is 9,000 or 3.95 $\log_{10}$ International Units/ampoule (see text).