Product Information Sheet for NR-3076

Murine Interferon Alpha (MuIFN-α)

Catalog No. NR-3076
This reagent is the property of the U.S. Government.

Lot (NIAID Catalog) No. Ga02-901-511

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: Murine Interferon Alpha (MuIFN-α)
NIAID Class: WHO International Standard
Research Reference Reagent Note (attached): No. 40
Titer: 16,000 International Units/ampoule
Molecular Weight:
28,000 daltons, primary component (99.5%)
36,000 daltons, minor component
Isoelectric Focusing: A major peak of activity at isoelectric point 7.4

Method of Preparation:
Tissue Culture System: Induced in L cells by Newcastle Disease Virus
Medium: Protein-free nutrient medium
Treatment: MuIFN-α separated from MuIFN-β by chromatography on controlled pore glass. Suspended in sodium phosphate 0.1 M, pH 7 with human serum albumin (1 mg/mL) and gelatin (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 28 hour period. Product is estimated to have unlimited stability at -20°C and -70°C

Purity:
Activity on Heterologous Cells: 200 Laboratory Units/mL in human A549 cells and 53 Laboratory Units/mL in rabbit RK-13 cells
Sterility: No evidence of bacterial or fungal contamination

Producer and Contract:
Medical College of Wisconsin

Citation:
Acknowledgment for publications should read "The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: Murine Interferon Alpha (MuIFN-α), NR-3076."

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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1. "Interferon Standards: A Memorandum." J. Biol. Stand. 7 (1979): 383–395. PubMed: 536379.

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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.
3. Grossberg, S. E. and G. J. Galasso. "Problems in Standardization: An Interferon Standards Committee Report." The Biology of the Interferon System. Eds. De Maeyer, E., G. Galasso, and H. Schekellens. Amsterdam: Elsevier/North Holland Biomedical Press, 1981. 19–22.
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RESEARCH REFERENCE REAGENT NOTE No. 40

Freeze-dried Reference Murine Interferon Alpha [MuIFN-α]
Catalog Number Ga02-901-511

RESEARCH RESOURCES SECTION
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, Maryland 20205
March 1987

Freeze-dried Reference Murine Interferon Alpha (Ga02-901-511)

Preparation: The interferon was produced by LEE Biomolecular Research Laboratories, Inc., San Diego, California. The interferon was induced in L cells in protein-free nutrient medium by Newcastle disease virus (NDV), and held at pH 3 for 2 weeks at 4°C to inactivate the NDV. The MuIFN-α was separated from the MuIFN-β by differential chromatography on controlled pore glass, and held at pH 3, 4°C, until fractions were pooled and freeze-dried. Two lots of the interferon were provided: lot number 83002 contained 7.4×10^5 International Units (IU)/ml, with a specific activity of 1.7×10^6 IU/mg, and was packaged as 700 KU (Cat. No. 22061); and lot number 83007 contained 1.8×10^6 IU/ml, with a specific activity of 2.7×10^6 IU/mg, and was packaged as 1.8 MU (Cat. No. 22061). The biological activities are those measured by the producer. Both lots were freeze-dried in 0.4 M glycine-HCl at pH 3.5.

Forty vials of lot 83002 and 4 vials of lot 83007 were used for the preparation of the reference reagent. This material was reconstituted, pooled, and supplemented as follows. The interferon was reconstituted with sterile distilled water, 1 ml/vial, and the contents of all vials were pooled. Each vial was rinsed with an additional 1.0 ml of sterile 0.1 M sodium phosphate buffer, pH 7, which was added to the pool. The pooled IFN preparation was aseptically diluted into ice-cold sterile buffer solution composed of 0.1 M sodium phosphate buffer, pH 7, supplemented with gelatin, to a final concentration of 5 mg/ml, and human serum albumin, (HSA) using 25% ''Buminate'' (Travenol) to a final concentration of 1 mg/ml. 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 consistency of the filling, determined gravimetrically, with 12 samples, was 1.0153 grams/vial, with a standard deviation of 0.0032 grams (coefficient of variation = 0.31). Ampoules were filled in groups of 19 and held on ice until 5 groups were filled which 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 the tips were heat-fused at atmospheric pressure. Each ampoule tip was dipped in neoprene solution to insure complete sealing. The last ampoule filled in each group of 19 was marked for testing of sterility and antiviral activity after freeze-drying. One randomly selected box of 144 ampoules, containing representatives of several groups from various stages of the filling and sealing procedures, was subjected to a test for the completeness of the seal. The ampoules were submerged in water containing a dye under a partial vacuum at room temperature, and inspected for the presence of liquid 20 minutes after they were returned to atmospheric pressure (according to World Health Organization recommendations[1]). 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 ampoules. 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, 1 mg/ml, and gelatin, 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, but repeated thawing and freezing may 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[2] 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[2], 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 0.5 years at 56°C, 3.8 years at 37°C, 25.9 years at 20°C, and 217.9 years at 4°C.

Test results: No bacteria or fungi were detected in 51 samples tested from the 155 different groups of ampoules composing the reference lot. The IFN used for freeze-drying was diluted to contain 1 mg of protein/ml (considering the product to have 6 mg/ml as 1 mg/ml HSA and 5 mg/ml gelatin) and characterized as follows: it was more than 99% inactivated by trypsin in 1 hr, 27% inactivated during heating at 56°C for up to 3 hr, and not inactivated during 48 hr of pH 2 dialysis at 4°C. The product was not neutralized by antisera to MuIFN-γ (prepared by E. Havell) but it was neutralized by anti-MuIFN-α/β serum (NIH G024-501-568). The IFN was composed primarily (99.5%) of a molecular size of 28,000 daltons, with a minor component of 36,000 daltons, as estimated by discontinuous gel electrophoresis in Laemmli buffers in 8-18% linear polyacrylamide gradients, by the producer. Analysis of MuIFN-α by isoelectric focusing revealed a major peak of activity with an isoelectric point of 7.4.

Potency was determined from the data contributed by seven international laboratories which had performed five or more titrations of the preparation (Table 1). Each laboratory used the method of their choice.

The geometric mean titer (GMT) calculated as the mean of the GMT values reported from each laboratory (total number of titrations = 54) was 4.005 log Laboratory Units (LU) (with a standard deviation, S.D., of 0.374 log corresponding to about 2.4-fold variation).

There was little activity on cells of heterologous species, with the following observed unadjusted titers obtained by the hemagglutination yield-reduction method[8] using encephalomyocarditis virus (EMCV): 200 LU with the human A549 lung carcinoma cell line, and 53 LU with the RK-13 rabbit kidney cell line.

Titer assignment: The assigned potency of Ga02-901-511 is 16,000 International Units (IU) (4.204 log IU). The assigned titer of the MuIFN-α NIH Reference Reagent Ga02-901-511 was derived from the test results of an international collaborative study by proportional relationship to the International Reference Preparation, Murine Interferon G002-904-511 having an assigned potency of 12,000 IU (4.08 log IU). Although the International

Reference G002-904-511 contains approximately 90% MuIFN-β. There is a significant correlation by linear regression analysis of the ratio of the GMTs of the Ga02-901-511 relative to the G002-904-511 for all the laboratories, justifying use of the proportional relationship in assignment of potency.

Use of Reference Interferon: The purpose of the MuIFN-α Reference Interferon Reagent is to provide a comparison of the sensitivities of bioassays that measure the antiviral activity of MuIFN-α in different laboratories. This preparation should be used only for the calibration of laboratory preparations of MuIFN-α which have dose-response curves parallel to that of the Reference Reagent[4-9]. Each laboratory should measure the MuIFN-α 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[4-7]. 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:

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

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

$$(2)\quad \frac{\text{lab std. IU}}{\text{lab std. observed LU}} \times \text{test sample observed LU} = \text{test sample IU}$$

It is important to recognize that the accuracy of estimation of the titer of a given samples depends largely upon the number of determinations done in spearate titrations. The range of expected mean titers for various numbers of titrations, based on the variance calculated for the results submitted in the collaborative assay, is presented in Table 2.

Table 2. Range of expected mean titers for a given number of titrations of the murine interferon alpha standard, Catalog number Ga02-901-511.

Number of titrations:	1	3	5	10	20
Range of expected mean titers:					
low	5,019	8,192	9,525	11,087	12,344
high	50,979	31,234	26,861	23,077	20,728
Magnitude of range (factor):	10.2	3.8	2.8	2.1	1.7
Range of expected log GMTs:					
low	3.70	3.91	3.98	4.04	4.09
high	4.71	4.49	4.43	4.36	4.32

References:

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Table 1. Summary of results of the international collaborative study of the murine interferon alpha reference preparation (NIH catalogue number Ga02-901-511)

Assay method	Observed LU/ml and variance within laboratories[a]							Summary of results
	1	2	3	4	5	6	7	All tests in all laboratories[b]
Number of titrations	6	5	8	5	5	5	5	
GMT (log)	4.510	3.821	4.182	3.609	3.531	4.006	3.377	4.005[c]
SD (log)	0.478	0.358	0.106	0.067	0.165	0.282	0.185	0.374

[a] The geometric mean titers (GMT) and standard deviations (SD) are based on titers calculated from the raw data provided by each laboratory.

[b] In this column the GMT and SD are based on the mean of the GMT values obtained for all laboratories.

[c] The assigned potency of Ga02-901-511, in relation to the International Reference Preparation of Mouse Interferon G002-904-511, is 16,000 or 4.204 $\log_{10}$ International Units (see text).