Product Information Sheet for NR-3077

Human Lymphoblastoid Interferon Alpha

Catalog No. NR-3077

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

Lot (NIAID Catalog) No. Ga23-901-532

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 Lymphoblastoid Interferon Alpha
NIAID Class: WHO International Standard
Research Reference Reagent Note (attached): No. 30
Titer: 25,000 International Units/ampoule
Molecular Weight: 15,500 daltons
Isoelectric Focusing: One major peak of activity at isoelectric point 5.8, with a shoulder at 6.2

Method of Preparation:

Tissue Culture System: Human lymphoblastoid Namalwa cell lines induced with Sendai virus
Treatment: Purified by serial chromatography at Wellcome Research Laboratories. Suspended in 0.1 M sodium phosphate buffer, pH 7 supplemented with 5 mg/mL human serum albumin
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: 70% of activity was lost during heating to 80°C; 81% as temperature reached 90°C. Product is estimated to have unlimited stability at -20°C and -70°C

Purity:

Activity on Heterologous Cells:
1.2×10^5 Laboratory Units/mL in human A549 cells
1.9×10^5 Laboratory Units/mL in bovine EBTr cells
7×10^4 Laboratory Units/mL in feline FEA cells
3.2×10^2 Laboratory Units/mL in murine 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 Lymphoblastoid Interferon Alpha, NR-3077."

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:

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government make any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:

This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

References:

1. Johnston, M. D., K. H. Fantes, and N. B. Finter. "Factors Influencing Production of Interferon by Human

Biodefense and Emerging Infections Research Resources Repository
P.O. Box 4137
Manassas, VA 20108-4137 USA
www.beiresources.org
800-359-7370
Fax: 703-365-2898
E-mail: contact@beiresources.org

Lymphoblastoid Cells." Adv. Exp. Med. Biol. 110 (1978): 61–74. PubMed: 215014.

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. Georgiades, J. A., M. P. Langford, G. J. Stanton, and H. M. Johnson. "Purification and Potentiation of Human Immune Interferon Activity." IRCS Medical Science 7 (1979): 559.
4. 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.
5. Jameson, P. and S. E. Grossberg. "Virus Yield-Reduction Assays for Interferon: Picornavirus Hemagglutination Measurements." Methods Enzymol. 78 (1981): 357–368. PubMed: 6173613.
6. Finter, N. B. "Large Scale Production of Human Interferon from Lymphoblastoid Cells." Tex. Rep. Biol. Med. 41 (1981-1982): 175–178. PubMed: 6184803.
7. World Health Organization. Interferon Therapy. WHO Technical Report Series No. 676.
8. World Health Organization. Standardization of Interferons, Annex to WHO Technical Report of Expert Committee on Biological Standardization. WHO Technical Report Series No. 687, 1983, pp. 35–60.
9. Grossberg, S. E., P. Jameson, and J. J. Sedmak. "Assay of Interferons." Handbook of Experimental Pharmacology, Volume 71. Eds. Came, P. and W. Carter. Berlin: Springer-Verlag, 1983. 23–43.

Support Provided by NIAID

Biodefense and Emerging Infections Research Resources Repository
P.O. Box 4137
Manassas, VA 20108-4137 USA
www.beiresources.org

800-359-7370
Fax: 703-365-2898
E-mail: contact@beiresources.org

NR-3077

RESEARCH REFERENCE REAGENT NOTE No. 30

Freeze-dried Reference Human Interferon Alpha [HuIFN-α (Namalwa/Sendai)]

Catalog Number Ga23-901-532

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

Research Reference Reagent Note No. 30

Freeze-dried Human Interferon Alpha (Namalwa/Sendai) Reference (Ga23-901-532)

Preparation: Human interferon alpha [HuIFN-α(Namalwa/Sendai)] was prepared by the Wellcome Research Laboratories, The Wellcome Foundation Ltd., Beckenham, England. Cultures of the human lymphoblastoid Namalwa cell line were infected with Sendai virus (1). After one day of incubation, the supernatant fluids were collected following centrifugation, and the IFN was purified by a series of differential precipitation (2) and chromatograhphic steps to obtain a purity of 87.4%, with a specific activity of 10^8 IU/mg, in a preparation composed of eight different alpha interferons as distinguished by physico-chemical characterization (1). After shipment to the Medical College of Wisconsin, the material was stored at -70°C. Subsequently, the sterile IFN preparation, containing 44.56 μg of HuIFN-α(Namalwa/Sendai), 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, containing 13.5 ng of HuIFN-α(Namalwa/Sendai), 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 25 preweighed vials (distributed throughout the fill), was 0.61 (coefficient of variation). Ampoules were filled in groups of 19, 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 19 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 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, 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 was tested twice by the linear non-isothermal accelerated degradation test (3) in which material is progessively heated from 50°C to 90°C over a 28-hour period. In replicate titrations of these two tests there was a range of inactivation from 0 to 90% between 50 and 80°C; but the observation that some of the degradation curves showed no loss of activity gave evidence that in spite of variability of

results, the IFN was sufficiently stable. From the results of the predictive multiple isothermal accelerated degradation test (3), involving storage of ampoules at 52°, 60°, 68°, and 76°C, with samples being removed at appropriate intervals over the course of 11 months, the product is estimated to be stable at -70°C for several decades. The length of time required to lose 1 log of activity at higher temperatures was estimated from these data to be 0.34 years at 56°C, 1.58 years at 37°C, 6.79 years at 20°C, 11.16 years at 4°C, and 24.42 years at -20°C, and 233 years at -70°C.

Test results: No mycoplasma, bacteria or fungi were detected in 43 samples tested from the 162 different groups of ampoules composing the reference lot. 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 tryspin in 1 hr, inactivated about 50% 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 HuIFN-γ (either provided by Irwin Braude, Meloy Labs, Springfield, VA, or prepared at MCW against purified HuIFN-γ), or by anti-HuIFN-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 one molecular size of 15,500 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 one major peak of activity at pH 5.8, with a shoulder at pH 6.2.

Potency was determined from the 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) (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 4.59 log units/ml/ampoule (with a standard deviation, S.D., 0.38 log corresponding to about 2.4-fold variation). Titration of the HuIFN-α(Namalwa/Sendai) by routinely used bioassays of different types with various cell-virus combinations, mostly dye-uptake measurements of cytolysis, gave GMT values ranging from 3.69 to 4.95 log units/ml, with a mean of 4.20 log units/ml (S.D., 0.53). 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): 1.2×10^5 Laboratory Units (LU)/ml in human A549 cells, 1.9×10^5 LU/ml in bovine EBTr cells, 7×10^4 LU/ml in feline FEA cells, and 320 LU/ml in murine L cells.

Titer assignment: The assigned titer of the HuIFN-α (Namalwa/Sendai) NIH Reference Reagent Ga23-901-530 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/ml (see Table 1). As a basis for expressing unitage of HuIFN-α (Namalwa/Sendai) in terms of IU established for MRC 69/19 HuIFN-α(Le), slopes of dose-response curves for HuIFN-α(Namalwa/Sendai) and MRC 69/19 were analyzed;

there was no significant difference in slopes for the two IFNs whether calculated from the 44 individual titrations (1.165 [S.D., 0.18] and 1.137 [S.D., 0.18, respectively) or from the average slopes observed in each of the eight laboratories (1.142 [S.D., 0.24] and 1.074 [S.D., 0.26], respectively). Therefore, the assigned potency of Ga23-901-532 is 25,000 International Units or 4.40 log IU/ampoule.

Use of Reference Interferon: The purpose of the HuIFN-α(Namalwa/Sendai) Reference Interferon Reagent is to provide a comparison of the sensitivities of bioassays that measure the antiviral activity of HuIFN-α(Namalwa/Sendai) in different laboratories. This preparation should be used only for the calibration of laboratory preparations of HuIFN-α (Namalwa/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 lymphoblastoid IFN preparation under test are 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(Namalwa/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:

1. Johnson, M.D., Fantes, K.H., and Finter, N.B.: Factors Influencing Production of Interferon by Human Lymphoblastoid Cells. Adv. Exper. Med. Biol. 110:61-74, 1978.

2. Finter, N.B.: Large Scale Production of Human Interferon from Lymphoblastoid Cells. Tex. Rep. Biol. and Med. 41:175-178, 1982.

3. Jameson, P., Greiff, D. and Grossberg, S.E.: Thermal Stability of Freeze-dried Mammalian Interferons: Analysis of Freeze-drying Conditions and Accelerated Storage Tests for Murine Interferon, Cryobiology 16:301-314. 1979.

4. Interferon Standards: A Memorandum (report to the World Health Organization on a Workshop on Interferon Standards, September 1978). J. Biol. Stand. 7:383-395, 1979.

5. Grossberg, S.E., Jameson, P., and Sedmak, J.J.: Assay of Interferons. In Handbook of Experimental Pharmacology, Vol. 71, P. Came and W. Carter (eds.), Springer-Verlag, Berlin, 1983, pp. 23-43.

6. Jameson, P. and Grossberg, S.E.: Virus Yield-reduction Assays for Interferon: Picornavirus Hemagglutination Measurements. Meth. Enzymol. 78:357-368, 1981.

7. Grossberg, S.E. and Galasso, G.J.: Problems in Standardization: An Interferon Standards Committee Report. In: The Biology of the Interferon System, E. DeMaeyer, G. Galasso, and H. Schellekens (eds.), Elsevier/North-Holland Biomedical Press, The Netherlands, 1981, pp. 19-22.

8. Interferon Therapy (Report of a World Health Organization Scientific Group), WHO Technical Report Series, No. 676, 1982.

9. Standardization of Interferons, Annex to WHO Report of Expert Committee on Biological Standardization, WHO Technical Report Series, No. 687, pp. 35-60, 1983.

Table 1. Summary of results of the international collaborative study of the Human Interferon Alpha (Namalwa/Sendai) Reference Preparation NIH catalog number Ga23-901-532

	Results obtained per laboratory								Summary per total	
Assay methods	#1	#2	#3	#4	#5	#6	#7	#8	labs[b]	test[c]
EMCV yield-reduction[a]										
Number of titers	10	5	2	6	8	5	5	3	8	44
GMT (log)	5.16	5.08	4.49	4.73	4.27	4.23	4.16	4.60	4.59*	4.64*
S.D. (log)	0.44	0.15	0.33	0.44	0.16	0.44	0.31	0.40	0.38	0.51
Other assay methods										
Number of titrations	24	5	NT	NT	7	6	5	5	6	
GMT (log)	3.69	4.70	-	-	4.57	4.95	3.80	3.99	4.28	
S.D. (log)	0.12	0.12	-	-	0.33	0.16	NA	0.11	0.52	

a/ The reference bioassay method measured the reduction in yield of encephalomyocarditis virus (EMVC) 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 0.35 log, corresponding to about 2.2-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 Ga23-901-532, in relation to the International Reference Preparation of Human Leukocyte Interferon 69/19, is 25,000 or 4.4 $\log_{10}$ International Units/ampoule (see text).