Product Information Sheet for NR-3080

Human Interferon Beta (HuIFN-β)

Catalog No. NR-3080

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

Lot (NIAID Catalog) No. Gb23-902-531

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 Interferon Beta (HuIFN-β)
NIAID Class: WHO International Standard
Research Reference Reagent Note (attached): No. 35
Titer: 15,000 International Units/ampoule
Molecular Weight: 22,000 daltons
Isoelectric focusing: A major peak of activity at isoelectric point 5.5

Method of Preparation:

Tissue Culture System: Produced in FS-4 human foreskin fibroblast cultures by super-induction with poly (I)-poly (C)
Treatment: Chromatography on controlled pore glass at Dr. Rentschler Arzneimittel GmbH Co. Suspended in 0.1 M sodium phosphate, pH 7 supplemented 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:
2.2×10^4 Laboratory Units/mL in human A549 cells
1.8×10^2 Laboratory Units/mL in murine L cells
8.25×10^2 Laboratory Units/mL in RK-13 cells
Sterility: No evidence of bacterial or fungal contamination

Producer and Contract:

Dr. Rentschler Arzneimittel GmbH Co. and the Medcial 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: Human Interferon Beta (HuIFN-β), NR-3080."

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. Havell, E. A. and J. Vilcek. "Production of High-Titered Interferon in Cultures of Human Diploid Cells." Antimicrob. Agents Chemother. 2 (1972): 476–484. PubMed: 4670440.
2. "Interferon Standards: A Memorandum." J. Biol. Stand. 7 (1979): 383–395. PubMed: 536379.

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

3. 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.
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. World Health Organization. Interferon Therapy. WHO Technical Report Series No. 676.
7. 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.
8. 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.
9. Grossberg, S. E., J. L. Taylor, R. E. Siebenlist, and P. Jameson. "Biological and Immunological Assays of Human Interferons." Manual of Clinical Laboratory Immunology. 3rd ed. Eds. Rose, N. R., H. Friedman, and J. L. Fahey. Washington, DC: American Society for Microbiology, 1986. 295–299. NLM ID: 8509431.
10. World Health Organization. Standardization of Interferons, Annex to WHO Expert Committee on Biological Standardization, 35th Report. WHO Technical Report Series 725, Geneva, 1985.
11. Campbell, P. J. "Biological Standards: Problems in Large Scale Production." International Symposium on Freeze-Drying of Biological Products. Eds. Cabasso, V. J. and R. H. Regamey. Basel: Karger, 1977. 355–363.
12. de Ley, M., et al. "Interferon Induced in Human Leukocytes by Mitogens: Production, Partial Purification and Characterization." Eur. J. Immunol. 10 (1980): 877–883. PubMed: 6161829.

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-3080

RESEARCH REFERENCE REAGENT NOTE No. 35

Freeze-dried Reference Human Interferon Beta [HuIFN-β]
Catalog Number Gb23-902-531

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

Freeze-dried Reference Human Interferon Beta (Gb23-902-531)

Preparation: Human fibroblast interferon beta [HuIFN-β] was produced in FS-4 human foreskin fibroblast cultures by superinduction with poly (I) poly (C)[1] by Dr. Rentschler Arzneimittel GmbH Co, 7958 Laupham, West Germany. It was originally provided to the National Cancer Institute of the National Institutes of Health (NIH) as freeze-dried preparations, lot 4, sent in October, 1977, and study number ''charge g'' sent in 1979. The first lot was stated to contain HuIFN-β concentrations of 8 x 10^5 IU/ml, and 10^6 IU/ml respectively, and protein contents of 9 and 6 mg/ml respectively. The specific activities of the two lots were 9 x 10^4, and 10^5 IU/mg respectively before human serum albumin was added as a stabilizer. The NIH sent 220 vials of the 1977 lot and 45 vials of the 1979 lot to the Medical College of Wisconsin for the production of an HuIFN-β standard reagent.

For the preparation of the reference reagent, the two lots of interferon were reconstituted, partially purified, pooled, and supplemented as follows. The interferon was reconstituted with sterile distilled water, 1 ml/vial, and the contents of all vials of a given lot were pooled, each vial was then rinsed with an additional 0.2 ml which was added to the pool. Each lot was purified independently by chromatography[2] through controlled pore glass (CPG) column, from which the IFN was eluted with 0.1 M HCl-KCl buffer, pH 2, containing gelatin, 5 mg/ml. Previous purification attempts resulted in products of acceptable purity but very poor stability during storage at either 4°C or -70°C for even brief periods; and the addition of gelatin during purification permitted optimal recovery of the IFN activity. The purified IFN was filter-sterilized through a 0.2 μm membrane and left at pH 2 for storage at 4°C while the two lots were titrated. They were combined immediately prior to freeze-drying, and human serum albumin (HSA) was added to a final concentration of 1 mg/ml, using a stock solution of 25% HSA (Travenol ''Buminate''). 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 1 mg/ml HSA and 5 mg/ml gelatin. 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 13 samples, was 1.0047 grams/vial, with a standard deviation of 0.0025 grams (coefficient of variation = 0.25). 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 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. All of these marked vials were subjected to a test for the completeness of the seal by submersion in water with 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[3]). 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[4] 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[4], 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 one log of activity at temperatures above freezing was estimated from these data to be 1.3 years at 56°C, 16.6 years at 37°C, 180.9 years at 20°C, and 2570 years at 4°C.

Test results: No bacteria or fungi were detected in 60 samples tested from the 154 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, 90% 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 HuIFN-γ (prepared at the Medical College of Wisconsin against purified HuIFN-γ), or by anti-HuIFN-α serum (NIH G026-502-568); but it was neutralized completely by anti-HuIFN-β serum (NIH G028-501-568). The IFN was composed of primarily one molecular size of 22,000 daltons, as estimated by sodium-dodecyl-sulfate polyacrylamide-gel electrophoresis in phosphate buffer by the method of Weber and Osborne. Analysis of HuIFN-β by isoelectric focusing revealed a major peak of activity with an isoelectric point of 5.5.

Potency was determined from the data contributed by seven international laboratories which had performed three or more titrations of the preparation using a microtiter reference bioassay technique[5] (Table 1). The reference bioassay involves the spectrophotometric measurement of the uptake of naphthol blue-black dye in cultures of the A549 line of human lung carcinoma cells infected with encephalomyocarditis virus (EMCV) after treatment with dilutions of the interferon samples. The endpoint is defined as the median dye uptake between optical density values for cultures that were not treated with interferon but were infected with EMCV (virus controls) and those that were not infected with EMCV (cell controls).

The geometric mean titer (GMT) calculated as the mean of the GMT values reported from each laboratory (total number of titrations = 131) was 3.8131 log Laboratory Units (LU) (with a standard deviation, S.D., of 0.368 log corresponding to about 2.3-fold variation). Six of the laboratories also

titrated the HuIFN-β by routinely used bioassays of different types with various cell-virus combinations; the resulting GMT values ranged from 3.31 to 4.17 log LU (based on a total of 50 determinations) with a mean of 3.73 log LU, S.D. 0.31 (Table 1).

There was little activity on cells of heterologous species. The following observed unadjusted titers were obtained by the hemagglutination yield-reduction method[6] using the GDVII strain of encephalomyocarditis virus with L cells, and EMCV with all other types of cells: 22,000 LU in human A549 cells, 180 LU in murine L cells, and 825 LU with RK-13 cells.

Titer assignment: The assigned potency of the HuIFN-β NIH Reference Reagent Gb23-902-531 is 15,000 international units (IU), or 4.176 log IU. This value is derived from the test results of an international collaborative study using the reference bioassay by proportional relationship to the International Reference Preparation, Human Fibroblast Interferon, G023-902-527 having an assigned potency of 10,000 IU.

Use of Reference Interferon: The purpose of the HuIFN-β Reference Interferon Reagent is to provide a comparison of the sensitivities of bioassays that measure the antiviral activity of HuIFN-β in different laboratories. This preparation should be used only for the calibration of laboratory preparations of HuIFN-β which have dose-response curves parallel to that of the Reference Reagent[7-12]. Each laboratory should measure the HuIFN-β 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 in accord with to recommendations by the World Health Organization[7-10]. 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 sample depends largely upon the number of determinations done in separate 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 human fibroblast interferon standard Gb23-902-531.

Number of titrations:	1	3	5	10	20
Range of expected mean titers:					
low	4,800	7,769	9,010	10,460	11,624
high	46,857	28,951	24,961	21,501	19,348
Magnitude of range (factor):	9.8	3.7	2.8	2.1	1.7
Range of expected log GMTs:					
low	3.68	3.89	3.95	4.02	4.07
high	4.67	4.46	4.39	4.33	4.29

References:

1. Havell, E.A., and Vilcek, J.: Production of high-titered interferon in cultures of human diploid cells. Antimicrob. Agents Chemother. 2:476-484.

2. de Ley, M., van Damme, J. Claeys, H., Weening, H. Heine, J.W., Billiau, A., Vermylen, C., and de Somer, P. Interferon induced in human leukocytes by mitogens: production, partial purification and characterization. Eur. J. Immunol. 10:877-883, 1980.

3. Campbell, P.J.: Biological standards: problems in large scale production. In International Symposium On Freeze-drying Of Biological Products, Cabasso, V.J., and Regamey, R.H., eds., Karger, Basel, 1977, pp. 355-363.

4. 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.

5. Grossberg, S.E., Taylor, J.L., Siebenlist, R.E., and Jameson, P.: Biological and immunological assays of human interferons. In: Manual of Clinical Immunol., Rose, N.R., Friedman, H., and Fahey, J.L., eds., 3rd edition., American Society for Microbiology, Washington, 1985, pp. 295-299.

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

7. Interferon Standards: A Memorandum (report to the World Health Organization on a Workshop on Interferon Standards, September 1978). Journal of Biological Standardization 7:383-395, 1979.

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

9. Standardization of Interferon, Annex to WHO Report of Expert Committee on Biological Standardization WHO Technical Report Series, No. 687, 1983

10. Standardization of Interferons, Annex to WHO expert Committee on Biological Standardization, 35th Report, Technical Report Series 725, WHO, Geneva, 1985.

11. 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.

12. 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.

Table 1. Summary of results of the international collaborative study of the human interferon beta reference preparation (NIH catalogue number Gb23-902-531)

Assay method	Observed LU/ml and variance within laboratories[b]							Summary of results: All tests in all laboratories[c]
	1	2	3	4	5	6	7	
Reference bioassay[a]								
Number of titrations	6	5	5	5	3	5	8	
GMT (log)	3.361	4.130	3.956	3.990	3.545	4.291	3.418	3.813[d]
SD (log)	0.138	0.114	0.269	0.085	0.162	0.410	0.243	0.368
Other assay methods								
Number of titrations	5	5	5	11	8	8	--[e]	
GMT (log)	3.806	4.167	3.937	3.673	3.513	3.308	--	3.734
SD (log)	0.106	0.167	0.087	0.176	0.190	0.218	--	0.306

[a] The reference bioassay method measured changes in absorbance of naphthol blue-black dye taken up by the human A549 cell line infected with encephalomyocarditis virus (EMCV). The EMCV was propagated in L cell cultures. A standard protocol (5) for the assay, as well as the EMCV, and the A549 and L cell lines were provided all participants by Dr. S. E. Grossberg's laboratory at the Medical College of Wisconsin.

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

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

[d] The assigned potency of Gb23-902-531, in relation to the International Reference Preparation of Human Fibroblast Interferon G023-902-531, is 15,000 or 4.176 $\log_{10}$ International Units (see text).

[e] A dash indicates that no titrations were done.