Product Information Sheet for NR-3086

Human Recombinant Interferon Gamma (rHuIFN-γ)

Catalog No. NR-3086

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

Lot (NIAID Catalog) No. Gxg01-902-535

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 Gamma (rHuIFN-γ)
NIAID Class: WHO International Standard
Research Reference Reagent Note (attached): No. 43
Titer: 80,000 International Units/ampoule

Method of Preparation:

Producer System: Extracted from plasmid-transformed *E. coli* cultures
Treatment: Purified by chromatography at Genetech. Suspended in sodium acetate 20 mM, pH 5.2 with human serum albumin (20 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 -70°C

Purity:

Activity on Heterologous Cells: None
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: Human Recombinant Interferon Gamma (rHuIFN-γ), NR-3086."

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

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. 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.
4. Jameson, P. and S. E. Grossberg. "Virus Yield-Reduction Assays for Interferon: Picornavirus Hemagglutination Measurements." Methods Enzymol. 78 (1981): 357–368. PubMed: 6173613.
5. World Health Organization. Interferon Therapy. WHO Technical Report Series No. 676.
6. 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.
7. 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.
8. Gray, P. W., et al. "Expression of Human Immune Interferon cDNA in *E. coli* and Monkey Cells." Nature 295 (1982): 503–508. PubMed 6173769.
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.

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. 43

Freeze-dried Recombinant Human Interferon Gamma Reference
Catalog Number (#Gxg01-902-535)

RESEARCH RESOURCES SECTION
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, Maryland 20205
February 1995

February 1995

Research Reference Reagent Note #43

Freeze-dried Recombinant Human Interferon Gamma Reference (#Gxg01-902-535)

Preparation: Recombinant human interferon gamma (rHuIFN-γ) was prepared at Genentech, South San Francisco, California. This interferon was derived from *E. coli* and highly purified by a series of chromatography steps, including cation exchange and gel filtration[1] and shipped to the Medical College of Wisconsin for further processing. The rHuIFN-γ was stored at 4°C while antiviral activity was being determined, then aseptically diluted into 20 mM sodium acetate pH 5.2 buffer containing human serum albumin and gelatin (in final concentration 20 mg/ml and 5 mg/ml, respectively), and dispensed by a high-precision Hamilton dispenser in 1.00 ml portions into glass ampoules for freeze-drying. The rHuIFN-γ was kept chilled throughout the dilution and dispensing process and placed immediately in the precooled freeze-drying chamber. After freezing, the ampoules were dried to a residual moisture of about 3%, backfilled with argon, and heat-sealed at atmospheric pressure; each ampoule tip was dipped in neoprene solution to ensure complete sealing. The last ampoule filled in each group of 24 was taken for testing of sterility and antiviral activity after freeze-drying. Ampoules are stored at -80°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 or in serum-containing culture medium used in the biological assay. Aliquots of the diluted IFN should preferably 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 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. Since HuIFN-γ is acid-labile, care should be taken to avoid exposure to CO_2, as may occur if screw-capped vials are stored on dry ice.

Stability: The freeze-dried reference reagent 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°, 60° and 68°C for periods up to 270 days, the time to lose one log in titer at 37°C is predicted to be at least 2 years; at 4°C at least 8 years; and at -20°C at least 15 years. At -70°C it is expected to have essentially unlimited stability.

Test results: No bacteria or fungi could be cultured from the preparation before freeze-drying or in the many samples tested after freeze-drying. Reproducibility of the fill (1.00 ml) dispensed with the Hamilton dispenser, as measured by the weight of liquid dispensed into 25 pre-weighted vials (distributed throughout the filling operation), was 0.5 (coefficient of variation). Ampoules were tested for defects in sealing by the methylene blue uptake method recommended by the World Health Organization and were found to be completely intact. Potency was determined from the data

contributed by 13 laboratories in 6 countries which had performed three or more titrations of the preparation [along with the International Standard for HuIFN-γ Gg23-901-530], using their routine method of bioassay. The observed geometric mean titer (GMT) calculated as the mean of the GMT values reported from each laboratory (total number of titrations = 72) was 4.95 log Laboratory Units (LU) (with a standard deviation, S.D., of 0.53 $\log_{10}$).

Table 1

Summary of results of the international collaborative study of the proposed international standard for recombinant human interferon gamma (NIH Catalog Number Gxg01-902-535)

	Observed LU/ml and variance within laboratories[a]										Summary of results of all tests in all laboratories[b]
	1	2	3	4	5	6	7	8	9	10	
Number of titrations	8	10	7	7	15	5	7	3	5	5	
GMT (log)	4.80	5.05	4.11	5.09	5.04	4.35	4.86	5.36	5.39	4.64	4.95[c]
SD (log)	0.34	0.16	0.25	0.23	0.09	0.28	0.24	0.15	0.13	0.06	0.53

a/ The geometric mean titers (GMT) and standard deviations (SD) are based on titers calculated at the Medical College of Wisconsin 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 Gxg01-902-535, in relation to the International Standard for Human Interferon Gamma Gg23-901-530, is 80,000 or 4.91 $\log_{10}$ International Units (see text).

Titer assignment: The assigned potency of the recombinant HuIFN-γ NIH Reference Reagent Gxg01-902-535 is 80,000 International Units (IU) (4.91 $\log_{10}$ IU) per ampoule. This value is derived from the bioassay test results of an international collaborative study by proportional relationship to the International Reference Preparation, Human Interferon Gamma, Gg23-901-530, having an assigned potency of 4,000 IU (3.60 $\log_{10}$ IU) per ampoule.

Use of Reference Interferon: The purpose of the Recombinant HuIFN-γ Reference Interferon Reagent is to provide a comparison of the sensitivities of bioassays that measure the antiviral activity of recombinant HuIFN-γ or other recombinant IFNs with dose-response curves parallel to that of the recombinant HuIFN-γ Reference Reagent[3-10] in different laboratories. Each laboratory should measure the recombinant HuIFN-γ 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 recommendation by the World Health Organization[5-8]. 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:

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

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

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

It is important to recognize that the precision 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 international the collaborative assay study, is presented in Table 2.

Table 2
Range of expected mean titers for a given number of titrations
of the recombinant human interferon gamma standard preparation
Gxg01-902-535

Number of titrations:		1	3	5	10	20
95%	low	25,924	39,448	44,892	51,130	56,058
Range	high	189,016	124,213	109,151	95,834	87,410
Magnitude of range		7.3	3.2	2.4	1.9	1.6

References:

1. Grey, T.W., Leung, D.W., Pennica, D., Yelverton, E., Najarian, R., Simonsen, C.C., Derynek, R., Sherwood, P.J., Wallace, D.M., Berger, S.L., Levinson, A.D., Goeddel, D.V.: Expression of Human Immune Interferon cDNA in *E. coli* and monkey cells. *Nature* (London) 295:503-508, 1982.

2. 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.

3. Grossberg, S.E., Taylor, J.L., Siebenlist, R.E., and Jameson, P.: Biological and immunological assays of human interferons. In: *Manual of Clinical Immunology*, 3rd. Edition, Rose, N.R., Friedman, H. and Fahey, J.L., eds., pp. 295-299, 1985.

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

5. 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.

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

7. Standardization of Interferons, Annex to WHO Expert Committee on Biological Standardization, 35th Report, Technical Report Series No. 725, WHO, Geneva, pp. 28-64, 1985.

8. Standardization of Interferons, (Report of a WHO Consultation) WHO Technical Report Series, No. 771, 37-87,1988.

9. 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.

10. Grossberg, S. E., and Sedmak, J.J.: The Assay of Interferons. In Volume 1, A. Billiau, ed., The Interferons and Their Inducers, 3rd edition, pages 189-216, N. Finter (ed.), North Holland Publishing Co., 1984.