Product Information Sheet for NR-3081

Murine Interferon Gamma (MuIFN-γ)

Catalog No. NR-3081

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Lot (NIAID Catalog) No. Gg02-901-533

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 Gamma (MuIFN-γ)
NIAID Class: WHO International Standard
Research Reference Reagent Note (attached): No. 42
Titer: 1,000 International Units/ampoule
Molecular Weight: 20,000 daltons and 40,000 daltons
Isoelectric focusing: A heterogeneous peak of activity within an isoelectric point range of 5.5 to 6.5

Method of Preparation:

Tissue Culture System: Mouse splenocytes stimulated by Concanavalin A and pretreated with mezerein prior to induction with lentil lectin
Medium: RPMI-1640 with 2.5% fetal bovine serum and 0.05 M 2-Mercaptoethanol
Treatment: Purified by chromatography on yeast RNA Sepharose. Suspended in 0.1 M sodium phosphate, 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 +4°C, -20°C and -70°C

Purity:

Activity on Heterologous Cells: None on human lung A549 cells or on rabbit kidney 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 Gamma (MuIFN-γ), NR-3081."

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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References:

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.
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. World Health Organization. Standardization of Interferons, Annex to WHO Expert Committee on Biological Standardization, 35th Report. WHO Technical Report Series 725, Geneva, 1985.
9. 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.
10. Taylor, J. L., et al. "Markedly Enhanced Production of Gamma Interferon in Murine T Lymphocytes Treated With Lentil Lectin and the Diterpene Ester, Mezerein." J. Interferon Res. 4 (1984): 315–327. PubMed: 6436398.

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NR-3081

RESEARCH REFERENCE REAGENT NOTE No. 42

Freeze-dried Reference Murine Interferon Gamma [MuIFN-γ]
Catalog Number Gg02-901-533

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

Freeze-dried Reference Murine Interferon Gamma (Gg02-901-533)

Preparation: The interferon (IFN) was produced at the Medical College of Wisconsin in mouse splenocytes stimulated to grow for four days by addition of Concanavalin A and pretreated with the phorbal ester, mezerein, for 3 hours prior to induction with lentil lectin from Lens culinaris[1]. Crude IFN was collected from the supernatant fluids one day later. All incubations were at 37°C. The IFN was concentrated by fractional ammonium sulfate precipitation and purified by single step chromatography on yeast RNA-sepharose eluted with 1 M NaCl in 0.02 M sodium phosphate buffer (SPB) at pH 7.2[1]. In preliminary tests, the specific activity of IFN-containing fractions was about 10^6 units/mg protein. For the production of large volumes the IFN for freeze-drying, the fractions expected to contain IFN were supplemented with gelatin (5 mg/ml) and human serum albumin (HSA) using 25% ''Buminate'' (Travenol) to a final concentration of 1 mg/ml. The fractions in the peak area were pooled, dialyzed first against 4 liters of 0.02 M SPB at pH 7.2 and then against 4 liters of 0.1 M SPB at pH 7. The dialyzed IFN was filter-sterilized through a 0.2 μm filter, and stored at 4°C. Smaller amounts of IFN prepared without the protein stabilizers were stored at -70°C after sterilzation and were supplemented with HSA and gelatin as above at the time of addition to the pool for freeze-drying.

For the preparation of the reference reagent, this pooled interferon was was aseptically diluted into ice-cold sterile buffer solution composed of 0.1 M sodium phosphate buffer, pH 7, supplemented with 5 mg/ml gelatin and HSA 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 18 samples was 1.00795 grams/vial, with a standard deviation of 0.0044 grams (coefficient of variation = 0.43). 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 box of 144 ampoules, containing samples from several stages of the filling and sealing procedures, was randomly selected for testing 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 (in accord with World Health Organization recommendations[2]). 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[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 4°C, -20°C and -70°C. The time predicted to lose 1 log of activity at higher temperatures was estimated from these data to be 0.64 years at 56°C, 32.2 years at 37°C, and 1318 years at 20°C.

Test results: No bacteria or fungi were detected in 40 samples tested from the 77 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, 73% inactivated during heating at 56°C for up to 3 hr, and 97% inactivated within 11 hours of pH 2 dialysis at 4°C. The product was not neutralized by antisera to MuIFN-α/β (NIH G024-501-568), but it was neutralized by anti-MuIFN-γ rabbit polyclonal and rat monoclonal antibodies (prepared by E. Havell). The IFN was composed of two molecular sizes, 20,000 and 40,000 daltons, as estimated by sodium-dodecyl-sulfate polyacrylamide-gel electrophoresis in phosphate buffer by the method of Weber and Osborne. Analysis of MuIFN-γ by isoelectric focusing revealed a heterogeneous peak of activity with an isoelectric point range of 5.5 - 6.5.

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 = 63) was 3.048 log Laboratory Units (LU) (with a standard deviation, S.D., of 0.558 log corresponding to about 3.6-fold variation).

There was no activity on cells of heterologous species by the hemagglutination yield-reduction method[4] using encephalomyocarditis virus (EMCV) in the human A549 lung carcinoma cell line and the RK-13 rabbit kidney cell line.

Titer assignment: The assigned potency of MuIFN-γ NIH Reference Reagent Gg02-901-533 is 1,000 International Units (IU) (3.0 log IU). This value was derived from the test results of an international collaborative study.

Use of Reference Interferon: The purpose of the MuIFN-γ Reference Interferon Reagent is to provide a comparison of the sensitivities of bioassays that measure the antiviral activity of MuIFN-γ in different laboratories. This preparation should be used only for the calibration of

laboratory preparations of MuIFN-γ which have dose response curves parallel to that of the Reference Reagent[5-10]. Each laboratory should measure the MuIFN-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 according to recommendations by the World Health Organization[5-8]. The number of Reference Units (RU)/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 determination done in separate titrations. The range of expected mean titers for various numbers of titrations, based upon 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 gamma standard Gg02-901-533.

Number of titrations:	1	3	5	10	20
Range of expected mean titers:					
low	215	412	503	615	709
high	4,652	2,429	1,989	1,626	1,410
Magnitude of range (factor):	21.6	5.9	4.0	2.6	2.0
Range of expected log GMTs:					
low	2.33	2.61	2.70	2.79	2.85
high	3.67	3.39	3.30	3.21	3.15

References:

1. Taylor, J.L., Sedmak, J.J., Jameson, P.J., Lin, Y.-G., and Grossberg, S.E.: Markedly enhanced production of gamma interferon in concanavalin A stimulated murine T-lymphocytes treated with lentil lectin and the phorbol ester, mezerein. J. Interferon Res., 4:315-327, 1984.

2. 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, pp. 355-363, 1977.

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

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

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

9. 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.

10. 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 murine interferon gamma reference preparation (NIH catalogue number Gg02-901-533)

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	9	5	5	5	7	
GMT (log)	3.896	2.658	3.533	2.959	3.304	2.299	2.686	3.048[c]
SD (log)	0.320	0.142	0.186	0.036	0.468	0.286	0.381	0.558

[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 Gg02-901-533 is 1,000 or 3.0 $\log_{10}$ International Units (see text).