

### Murine Interferon Beta (MuIFN- $\beta$ )

#### Catalog No. NR-3079

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

#### Lot (NIAID Catalog) No. Gb02-902-511

**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 Beta (MuIFN- $\beta$ )

NIAID Class: WHO International Standard

Research Reference Reagent Note (attached): No. 41

Titer: 15,000 International Units/ampoule

Molecular Weight: 36,000 daltons

Isoelectric Focusing: A major peak of activity at isoelectric point 7.2

#### Method of Preparation:

Tissue Culture System: Induced in L cells by Newcastle Disease Virus

Medium: Protein-free nutrient medium

Treatment: Purified by chromatography on controlled pore glass. 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 -20°C and -70°C

#### Purity:

Activity on Heterologous Cells: None on human lung A549 cell line and rabbit kidney RK-13 cell line

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 Beta (MuIFN- $\beta$ ), NR-3079."

#### 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/bmb15/bmb15toc.htm](http://www.cdc.gov/od/ohs/biosfty/bmb15/bmb15toc.htm).

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#### 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.
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, 35<sup>th</sup> 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.

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RESEARCH REFERENCE REAGENT NOTE No. 41

Freeze-dried Reference Murine Interferon Beta [MuIFN- $\beta$ ]  
Catalog Number Gb02-902-511

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

Freeze-dried Reference Murine Interferon Beta (Gb02-902-511)

Preparation: The interferon was produced by LEE Biomolecular Research Laboratories, Inc., San Diego, California. Interferon was induced in L cells in protein-free nutrient medium by Newcastle disease virus (NDV) and held at pH 3 for two weeks at 4°C to inactivate the NDV. The MuIFN- $\beta$  was purified by differential chromatography on controlled pore glass. The lot (number 82014) provided contained  $1.7 \times 10^7$  International Units (IU)/ml, with a specific activity of  $7.6 \times 10^7$  IU/mg, and was packaged as 12 MU (LEE Biomolecular Cat. No. 20171). The biological activities are those measured by the producer.

Seven vials of lot 82014, which had been freeze-dried in 0.4 M glycine-HCl at 3.5 were used for the preparation of the reference reagent. This interferon was reconstituted, pooled, and supplemented as follows. One vial of the interferon was reconstituted with one ml of sterile distilled water, and diluted 1:10 in sterile, complete freeze-drying buffer for titration prior to the day of freeze-drying. On the day of freeze-drying, the other six vials were reconstituted with complete freeze-drying buffer, 1 ml/vial, and the contents of all vials were pooled. Each vial was rinsed with an additional 1.0 ml of complete buffer, which was added to the pool. The pooled IFN preparation was aseptically diluted into ice-cold sterile complete buffer solution composed of 0.1 M sodium phosphate buffer, pH 7, supplemented with 5 mg/ml gelatin, 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 0.9865 grams/vial, with a standard deviation of 0.007 grams (coefficient of variation = 0.71). 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 ampoules representing 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 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<sup>1</sup>). 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  $-70^{\circ}\text{C}$  in volumes each sufficient for a single titration. It may be possible to store enough material in a single container at  $-70^{\circ}\text{C}$  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  $-70^{\circ}\text{C}$  or lower.

Stability: The freeze-dried reference preparation did not lose any activity in the linear non-isothermal accelerated degradation test<sup>2</sup> in which material is progressively heated from  $50^{\circ}\text{C}$  to  $90^{\circ}\text{C}$  over a 28-hour period. From the results of the predictive multiple isothermal accelerated degradation test<sup>2</sup>, involving storage at  $52^{\circ}\text{C}$ ,  $60^{\circ}\text{C}$ ,  $68^{\circ}\text{C}$ , and  $76^{\circ}\text{C}$  for periods up to 1 year, the product is estimated to have unlimited stability at  $-20^{\circ}\text{C}$  and  $-70^{\circ}\text{C}$ . The time predicted to lose 1 log of activity at temperatures above freezing was estimated from these data to be 1.7 years at  $56^{\circ}\text{C}$ , 12.4 years at  $37^{\circ}\text{C}$ , 82.5 years at  $20^{\circ}\text{C}$ , and 675 years at  $4^{\circ}\text{C}$ .

Test results: No bacteria or fungi were detected in 64 samples tested from the 158 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, 50% inactivated during heating at  $56^{\circ}\text{C}$  for up to 3 hr, and not inactivated during 48 hr of pH 2 dialysis at  $4^{\circ}\text{C}$ . The product was not neutralized by antisera to MuIFN- $\gamma$  (prepared by E. Havell), but it was neutralized by anti-MuIFN- $\beta$  serum (NIH G024-501-568). The IFN was composed primarily (99.5%) of one molecular size of 36,000 daltons, as estimated by discontinuous gel electrophoresis in Laemmli buffers with 8-18% linear polyacrylamide gradients by the producer. Analysis of MuIFN- $\beta$  by isoelectric focusing revealed a major peak of activity with an isoelectric point of 7.2.

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 = 55) was 3.974 log Laboratory Units (LU) (with a standard deviation, S.D., of 0.363 log corresponding to about 2.4-fold variation).

There was no activity on cells of heterologous species as measured by the hemagglutination yield-reduction method<sup>3</sup> 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- $\beta$  NIH Reference Reagent Gb02-902-511 is 15,000 International Units (IU) (4.176 log IU). This value 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 (4.079 log).

Use of Reference Interferon: The purpose of the MuIFN- $\beta$  Reference Interferon Reagent is to provide a comparison of the sensitivities of bioassays that measure the antiviral activity of MuIFN- $\beta$  in different

laboratories. This preparation should be used only for the calibration of laboratory preparations of MuIFN- $\beta$  which have dose response curves parallel to that of the Reference Reagent<sup>4-6</sup>. Each laboratory should measure the MuIFN- $\beta$  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<sup>4-7</sup>. 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 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 murine interferon beta standard Gb02-902-511.

Number of titrations:	1	3	5	10	20
Range of expected mean titers:					
low	6,543	9,290	10,349	11,537	12,458
high	34,374	24,209	21,732	19,495	18,053
Magnitude of range (factor):	5.3	2.6	2.1	1.7	1.4
range of expected log GMTs:					
low	3.82	3.97	4.01	4.06	4.10
high	4.54	4.38	4.34	4.29	4.26

## References:

1. 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.
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Table 1. Summary of results of the international collaborative study of the murine interferon beta reference preparation (NIH catalogue number Gb02-902-511)

Assay method	Observed LU/ml and variance within laboratories <sup>a/</sup>							Summary of results All tests in all laboratories <sup>b/</sup>
	1	2	3	4	5	6	7	
Number of titrations	6	5	9	5	5	5	5	
GMT (log)	4.348	4.069	4.204	3.730	3.569	3.524	4.377	3.974 <sup>c/</sup>
SD (log)	0.384	0.225	0.116	0.054	0.135	0.246	0.071	0.363

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

<sup>b/</sup>In this column the GMT and SD are based on the mean of the GMT values obtained for all laboratories.

<sup>c/</sup>The assigned potency of Gb02-902-511, in relation to the International Reference Preparation of Mouse Interferon G002-904-511, is 15,000 or 4.176 log<sub>10</sub> International Units (see text).