Product Information Sheet for NR-3085

Human Recombinant Interferon Beta$_{ser17}$ (rHuIFN-β_{ser})

Catalog No. NR-3085

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

Lot (NIAID Catalog) No. Gxb02-901-535

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 Beta$_{ser17}$ (rHuIFN-β_{ser})
NIAID Class: WHO International Standard
Research Reference Reagent Note (attached): No. 37
Titer: 6,000 International Units/ampoule
Molecular Weight : 19,000 daltons

Method of Preparation:

Producer System: Plasmid transformed *E. coli*
Treatment: Purified by precipitation, chromatography and diafiltration at Cetus Corp. 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: About 150 Laboratory Units/mL in mouse L cells
Sterility: No evidence of bacterial or fungal contamination

Producer and Contract:

Triton, Cetus and the 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 Beta$_{ser17}$ (rHuIFN-β_{ser}), NR-3085."

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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NR-3085

RESEARCH REFERENCE REAGENT NOTE No. 37

Freeze-dried Reference Human Recombinant Interferon Beta/ser [HuIFN-β/ser]
Catalog Number Gxb02-901-535

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

Freeze-dried Reference Human Recombinant Interferon Beta/ser (Gxb02-901-535)

Preparation: Human recombinant interferon beta/ser [HuIFN-β/ser] was produced by Cetus Corporation, 1400 Fifty-Third Street, Emeryville, CA 94608, as lot BP-126, in which they measured 5 x 10^7 IU/ml. The IFN was extracted from plasmid transformed Escherchia coli cultures and purified by a series of procedures involving precipitation, molecular exclusion chromatography and diafiltration[1-3]. The material was supplied as a freeze-dried preparation containing 0.25 mg IFN, with 12.5 mg human serum albumin (HSA), and 12.5 mg dextrose per vial, corresponding to a reconstituted volume of 1 ml. The specific activity of the IFN was therefore 2 x 10^8 IU/mg before addition of HSA as a stabilizing agent. Two vials of this lot were used for the production of this standard. Because of the high specific activity of this IFN, no further purification was required.

For the preparation of the reference reagent, this interferon was reconstituted in sterile distilled water and diluted directly into ice-cold, sterile 0.1 M sodium phosphate buffer at pH 7, supplemented with 1 mg/ml HSA (from 25% ''Buminate'' by Travenol), 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 12 samples taken at intervals throughout the process, was 1.0094 grams/vial, with a standard deviation of 0.0014 grams (coefficient of variation = 0.14). 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. All of these marked vials were subjected to a test for the completeness of the seal by submersion in water with 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 (according to World Health Organization recommendation[4]). All of the vials tested were found to be completely sealed. 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[5] 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[5], 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 2.1 years at 56°C, 29.6 years at 37°C, 368.3 years at 20°C, and 6067 years at 4°C.

Test results: No bacteria or fungi were detected in 63 samples tested from the 152 different groups of ampoules composing the reference lot. The physico-chemical properties of the IFN were determined by Cetus Corporation[2,3]. The amino acid sequence of the HuIFN-β/ser differs from that of natural HuIFN-β by having a serine instead of a cysteine at position 17. The molecular weight estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis was 19,000. Antiviral activity of HuIFN-β/ser was neutralized completely by anti-HuIFN-β serum (from Dr. C.Y. Tan) and by anti-HuIFN-β/ser serum (from Cetus Corporation) but it was not neutralized by antisera to HuIFN-γ (prepared at The Medical College of Wisconsin against purified HuIFN-γ), by anti-HuIFN-α(Ly) serum (NIH G030-501-533) or by anti-HuIFN-α(Le) serum (NIH G026-502-568).

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[6]. 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 = 133) was 3.421 log Laboratory Units (LU) (with a standard deviation, S.D., of 0.244 log corresponding to about 1.8-fold variation). Six of the laboratories also titrated the HuIFN-β/ser by routinely used bioassays of differert types with various cell-virus combinations, with resulting GMT values ranging from 3.056 to 3.83 log LU, with a mean of 3.45 log LU, S.D. = 0.350.

There was little activity on cells of heterologous species, with 150 units/ml (observed unadjusted titer) in murine L cells with the GDVII strain of encephalomyelitis virus detected by the hemagglutination yield-reduction method[7].

Titer assignment: The assigned potency of Gxb02-901-535 is 6,000 International Units (IU) (3.778 log IU). This value of the HuIFN-β/ser NIH Reference Reagent Gxb02-901-535 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/ser Reference Interferon Reagent is to provide a comparison of the sensitivities of bioassays that measure the antiviral activity of HuIFN-β/ser, or other recombinant β-IFNs with dose-response curves similar to that of the HuIFN-β/ser, in different laboratories by conversion to international units[6-13]. Each laboratory should measure the HuIFN-β/ser 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[8-11]. 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, when five or more titrations are done.

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

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 beta/ser interferon standard Gxb02--901-535.

Number of titrations:	1	3	5	10	20
Range of expected mean titers:					
low	2,065	3,241	3,723	4,281	4,726
high	17,421	11,101	9,663	8,403	7,613
Magnitude of range (factor):	8.4	3.4	2.6	2.0	1.6
Range of expected log GMTs:					
low	3.31	3.51	3.57	3.63	3.67
high	4.24	4 05	3.99	3.92	3.88

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Table 1. Summary of results of the international collaborative study of the human recombinant interferon beta/ser reference preparation (NIH catalogue number Gxb02-901-535)

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	9	5	5	3	5	5	
GMT (log)	3.253	3.712	3.438	3.449	3.009	3.693	3.395	3.421[d]
SD (log)	0.175	0.206	0.080	0.321	0.290	0.339	0.131	0.245
Other assay methods								
Number of titrations	4	9	5	9	8	5	--[e]	
GMT (log)	3.659	3.800	3.827	3.056	3.131	3.236	--	3.452
SD (log)	0.113	0.088	0.149	0.215	0.118	0.276	--	0.350

[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 (6) 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 Gxb02-901-535, in relation to the International Reference Preparation of Human Fibroblast Interferon G023-902-531, is 6,000 or 3.778 $\log_{10}$ International Units (see text).

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