

### Polyclonal Anti-Human Interferon Beta (antiserum, Human)

#### Catalog No. NR-3073

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#### Lot (NIAID Catalog) No. G038-501-572

#### 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: Polyclonal antiserum

Host: Human

Immunizing Antigen: Interferon beta (natural human fibroblast)

NIAID Class: WHO International Reference Reagent

Research Reference Reagent Note (attached): No. 45

Adjuvant used: None

#### Material Provided/Storage:

Composition: Lyophilized

Original Volume: 1.0 mL

Storage Temperature: 4°C or colder

Reconstitution: 1.0 mL sterile distilled water

#### Functional Activity:

Neutralizing Titer: 1:1700 against 10 Laboratory Units of  
human interferon beta

Antibody Cross-Reactivity: No neutralizing antibody was  
detected against human interferon  $\alpha$  or  $\gamma$

#### Purity:

Sterility: No bacteria or fungi were cultured from the  
preparation before or after freeze-drying

#### Producer and Contract:

Bulk serum provided by Dr. Peter von Wussow, Medizinische  
Hochschule, Hannover, Germany. Characterization and  
freeze-drying by 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: Polyclonal Anti-Human Interferon Beta (antiserum,  
Human), NR-3073."

#### 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](http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm).

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#### References:

1. 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.
2. 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.
3. 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.

4. Kawade, Y. "Quantitation of Neutralization of Interferon by Antibody." Methods Enzymol. 119 (1986): 558–573. PubMed: 2429157.
5. "Human Antibodies to Interferons: Report of a National Institutes of Health Workshop Held 25–26 July 1988 in Bethesda, MD." J. Interferon Res. 8 (1988): v–vii.

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**RESEARCH REFERENCE REAGENT NOTE No. 45**

**Freeze-dried Human Anti-Human Interferon-Beta Antibody Reference  
Catalog Number G038-501-572**

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

### Freeze-dried Human Anti-Human Interferon-Beta Antibody Reference (G038-501-572)

Preparation: Approximately 1.8 liters of human plasma were supplied by Dr. Wieland Wolf at Bioferon in Laupheim, Germany. The plasma was obtained from a 47-year-old female diagnosed with malignant melanoma for which she was given natural (fibroblast) human interferon beta therapy. She was subsequently noted to have circulating antibodies to human natural interferon- $\beta$ , and her plasma was then collected during three sessions of plasmapheresis. The plasma was converted into serum by addition of 3 units per ml topostasine (Hoffmann-LaRoche), incubated for one hour at 37°C and then 24 hours at 4°C, after which the plasma was centrifuged, and the final serum preparation stored at -20°C. The serum was shipped frozen to the Medical College of Wisconsin. Further characterization and freeze-drying were performed to satisfy the recommendations of the World Health Organization and of an international workshop on human antibodies to interferons that took place at the National Institutes of Health 25-26 July 1988 in Bethesda, Maryland USA<sup>1-3</sup>.

The serum was diluted 1:1.5 in sterile isotonic saline such that the expected final titer would be in the range of 1:5,000, measured against 10 LU/ml of HuIFN- $\beta$ <sup>(1-4)</sup>. The diluted serum was then dispensed with a high-precision Hamilton dispenser in 1.00-ml portions into glass ampoules for freeze-drying. The serum was kept on ice throughout the process of dilution and dispensing process and was placed immediately into the pre-cooled, freeze-drying chamber. After freezing at -30°C, the ampoules were dried to a residual moisture of about 3%, backfilled with argon, and sealed by fusion of the glass 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 neutralizing antibody activity after freeze-drying. 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 taken to avoid loss of any material in the neck or stem of the ampoules. Each 1.0 ml suspension constitutes the undiluted material. The antiserum should be heated at 56°C for 30 minutes to inactivate complement components prior to its use in the neutralization bioassay. Small portions of the reconstituted antibody may be stored at -70°C undiluted, or diluted in protein-containing solution. Frequent freezing and thawing should be avoided.

Stability: In the linear non-isothermal accelerated degradation test in which material is progressively heated from 40°C to 90°C over a 28-hour period<sup>2-5</sup> the freeze-dried reference reagent did not lose any activity in the samples taken at 50°, 60°, and 70°C, with 7.1% activity remaining at 80°C. The freeze-dried reference reagent did not lose any activity in samples stored at 37°C for 2 months, at 52°C for 1 month, or at 60°C for 0.5 month; samples stored at 52°C for 1.5 months and 60°C for 1 month had 2.5% and 13% activity remaining respectively.

Test results: The serum was demonstrated to be negative for antibodies to human immunodeficiency virus-1, human T-lymphotropic virus-1, and hepatitis C virus. The serum was negative for hepatitis B virus surface antigen and for antibodies to HBV surface and core antigens. Antibody to hepatitis A virus (HAV) was detected, but HAV IgM antibody studies did not suggest

recent infection with HAV. The rapid reagin test for syphilis was negative. (Immunological tests for HIV-1, HTLV-I, Hepatitis A and B were done at the Wisconsin State Laboratory of Hygiene in Madison; testing of anti-hepatitis C antibodies was done at the Milwaukee County Medical Complex clinical laboratory). Immunoglobulin G was purified from a sample of the original serum by selective elution from a protein A column (Bio-Rad). Based on the dilution of the serum dispensed, each ampoule contains 3.96 mg of IgG. The calculated specific activity is a neutralizing potency of 1:439 per mg of IgG per ampoule; thus, 2.278  $\mu$ g of IgG in this antiserum preparation can neutralize 10 units of natural HuIFN- $\beta$ . No bacteria or fungi were 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 7 pre-weighed vials (distributed throughout the fill), was 0.0116 (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 interferon neutralization data contributed by fourteen international laboratories. Each laboratory was asked to perform five or more titrations of the preparation, using their routine bioassay against natural human (fibroblast) and human recombinant beta interferon (ser<sub>17</sub>). Titers are expressed as the reciprocal of the dilution of serum which reduces 10 Laboratory Units of interferon per ml final concentration to one LU/ml, as previously recommended<sup>1-4</sup>. Data for the neutralization of both HuIFN- $\beta$  preparations are shown in Table 1. No detectable neutralizing antibody was demonstrable against HuIFN- $\alpha$  or - $\gamma$ .

Titer assignment: The neutralization of the anti-HuIFN- $\beta$  antibody NIH Reference Reagent G038-501-572 can be assigned on the basis of its reduction from 10 Laboratory Units (LU)/ml to 1 LU/ml of HuIFN- $\beta$  (Gb23-902-531) as 1700 (-3.24 log<sub>10</sub>).

Use of Reference Anti-IFN Antibody: The purpose of the anti-HuIFN- $\beta$  reference antibody reagent is to help laboratories as a guide to the validation of their neutralization bioassay to permit the appropriate comparison of neutralization results reported. Each laboratory should measure the titer of the anti-HuIFN- $\beta$  reference reagent against 10 LU/ml of natural (fibroblast) HuIFN- $\beta$  as well as the HuIFN- $\beta$  preparation(s) of interest simultaneously with an internal laboratory standard. Five or more titrations should be done on separate occasions. The observed reciprocal of the geometric mean titer of the antibody dilution (or the negative logarithm) should be reported with its standard deviation, along with the assigned titer of the anti-HuIFN- $\beta$  Reference Reagent G038-501-572, in accord with recommendations by the World Health Organization, that is, the dilution that reduces 10 LU/ml to 1 LU/ml without further correction; the results should not be reported in international neutralizing units or such designations. 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.



## References:

1. Human Antibodies to Interferons: Report of a National Institutes of Health Workshop Held 25-26 July 1988 in Bethesda, MD, *J. Interferon Research*, 8:v-vii, No. 6, December 1988.
2. Standardization of Interferons, Annex to WHO Report of Expert Committee on Biological Standardization WHO Technical Report Series, No. 687, pp. 35-60, 1983.
3. Standardization of Interferon, Annex to WHO Report of Expert Committee on Biological Standardization WHO Technical Report Series, No. 725, pp. 28-64, 1985.
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5. 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.

**Table 1**

Summary of interferon neutralization data from the international collaborative study of proposed international reference human anti-HuIFN- $\beta$  antiserum (G038-501-572)

HuIFN- $\beta$ antigen	Reciprocal of log <sub>10</sub> titer and variance within 12 laboratories obtained with the indicated interferon antigen (number of titrations)									
	1	2	3	4	5	6	7	8	9	10
Fibroblast Gb23-902-531	3.76 ± 0.36 (10)	3.27 ± 0.18 (11)	3.29 ± 0.18 (4)	3.09 ± 0.20 (5)	2.85 ± 0.21 (11)					
$\beta$ ser17 Gxb-02-901-535	3.65 ± 0.45 (9)	2.67 ± 0.18 (11)	-----	2.56 ± 0.18 (5)	2.30 ± 0.36 (11)					
Fibroblast Gb23-902-531	3.02 ± 0.12 (4)	3.38 ± 0.19 (8)	2.59 ± 0.19 (3)	3.34 ± 0.14 (5)	3.31 ± 0.08 (5)					
$\beta$ ser17 Gxb-02-901-535	2.43 ± 0.03 (4)	3.12 ± 0.13 (6)	2.36 ± 0.22 (3)	2.47 ± 0.11 (5)	2.83 ± 0.15 (5)					
	11	12	log <sub>10</sub> GMT <sup>a/</sup> — GMT <sup>a/</sup>							
Fibroblast Gb23-902-531	3.93 ± 0.16 (5)	3.02 ± 0.13 (5)	n = 12 (76)	1700						
			3.24 ± 0.34							
$\beta$ ser17 Gxb-02-901-535	-----	2.72 ± 0.13 (5)	n = 10 (64)	500						
			2.71 ± 0.44							

<sup>a/</sup> Summary of results of all tests in all laboratories as the geometric mean titer (GMT) and standard deviations