

### Polyclonal Anti-Human Interferon Alpha-2b (antiserum, Human)

#### Catalog No. NR-3072

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#### Lot (NIAID Catalog) No. G037-501-572

#### 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: Recombinant interferon alpha-2b

NIAID Class: WHO International Reference Reagent

Research Reference Reagent Note (attached): No. 44

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: 1:9500 against 10 Laboratory Units of  
human interferon alpha-2b

Antibody Cross-Reactivity: No detectable activity against  
natural human interferon  $\beta$  or recombinant human  
interferon  $\beta_{\text{Ser17}}$

#### Purity:

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

#### Producer and Contract:

Bulk serum provided by Dr. Wieland Wolf of Bioferon,  
Laupheim, 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 Alpha-2b (antiserum,  
Human), NR-3072."

#### 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. 44**

**Freeze-dried Human Anti-Human Interferon-Alpha Antibody Reference  
Catalog Number G037-501-572**

**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-Alpha Antibody Reference (G037-501-572)

Preparation: Two liters of human plasma were supplied by Dr. Peter von Wussow of the Medizinische Hochschule Hannover, Germany. The plasma was obtained from a 24-year-old female diagnosed with metastatic malignant melanoma for which she was given  $3 \times 10^6$  IU/week of recombinant interferon  $\alpha$ -2b subcutaneously for four weeks without major side-effects. During treatment her disease progressed. One month after interferon therapy began, her serum was noted to have a high titer of anti-interferon- $\alpha$  antibodies. Her plasma was subsequently collected by plasmapheresis over a period of three months; during the four months prior to beginning plasmapheresis the patient did not suffer clinically obvious infections. The plasma was converted into serum by the addition of 3,000 units topostasine (Hoffmann-LaRoche) per 100 ml plasma, after which it was incubated for 24 hours at room temperature, centrifuged, and the pellet discarded. The supernatant fluid was incubated at  $+4^\circ\text{C}$  for 3 weeks and centrifuged daily to remove additional particulate material. The final serum preparation was stored at  $-80^\circ\text{C}$  and 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</sup>.

The serum was diluted 1:1.78 in sterile isotonic saline such that the expected final titer would be in the range of 1:10,000, as measured against 10 Laboratory Units (LU)/ml of HuIFN- $\alpha 2^{(1-4)}$ . 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 and was placed immediately into the pre-cooled, freeze-drying chamber. After freezing at  $-30^\circ\text{C}$ , 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  $-70^\circ\text{C}$  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 ampoule. Each 1.0-ml suspension constitutes the undiluted material. The antiserum should be heated at  $56^\circ\text{C}$  for 30 minutes to inactivate heat-labile complement components prior to its use in the neutralization bioassay. Small portions of the reconstituted antibody may be stored at  $-70^\circ\text{C}$  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  $40^\circ\text{C}$  to  $90^\circ\text{C}$  over a 28-hour period<sup>2,3</sup>, the freeze-dried reference reagent did not lose any activity in the samples taken at  $50^\circ\text{C}$ ,  $60^\circ\text{C}$ , and  $70^\circ\text{C}$ , with 3.8% activity remaining at  $80^\circ\text{C}$ . The freeze-dried reference reagent did not lose any activity in samples stored at  $37^\circ\text{C}$  for 2 months, at  $52^\circ\text{C}$  for 1 month, or at  $60^\circ\text{C}$  for 0.5 month; samples stored at  $52^\circ\text{C}$  for 1.5 months and  $60^\circ\text{C}$  for 1 month had 22% and 0.5% activity remaining, respectively. It is anticipated that the reference reagent will have essentially unlimited stability at  $-70^\circ\text{C}$ .

Test results: The serum was demonstrated to be negative for antibodies to human immunodeficiency virus-1, human T-lymphotropic virus-I, and hepatitis C viruses. The serum was negative for hepatitis B virus surface antigen and for antibody to HBV core antigen but positive for antibody to HBV surface antigen indicating post-convalescence long after HBV infection. Antibody to hepatitis A virus 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-I, HTLV-I, Hepatitis A and B were performed at the Wisconsin State Laboratory of Hygiene in Madison; testing for anti-hepatitis C antibodies and syphilis 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 3.629 mg of IgG. The calculated specific activity is a neutralizing potency of 1:2673 per mg of IgG per ampoule; thus, 0.374  $\mu$ g of IgG in this antiserum preparation can neutralize 10 units of HuIFN- $\alpha$ 2b. 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 eleven pre-weighed vials (distributed throughout the fill), was 0.013 (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 laboratories (in seven countries). Each laboratory performed five or more titrations of the preparation, using their routine bioassay against seven recombinant or natural interferon- $\alpha$  preparations. 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>14</sup>. Data for the neutralization of the seven HuIFN- $\alpha$  preparations are shown in Table 1. No detectable neutralizing antibody was demonstrable against natural HuIFN- $\beta$  (Gb23-902-531) or recombinant HuIFN- $\beta$ ser<sub>17</sub> (Gxb02-901-535).

Titer assignment: The neutralization potency of the anti-HuIFN- $\alpha$  antibody NIH Reference Reagent G037-501-572 can be assigned as 1: 9500 ( $-3.95 \log_{10}$ ) for HuIFN- $\alpha$ 2 subtypes on the basis of its reduction from 10 Laboratory Units (LU)/ml to 1 LU/ml of HuIFN- $\alpha$ 2, as recommended by a WHO Committee (in press 1994). The potency values as the reciprocal of the titers assayed against other HuIFN- $\alpha$  materials are those reported as the final geometric mean titer (GMT) listed in Table 1, which summarizes the results of the international collaborative study.

Use of Reference Anti-IFN Antibody: The purpose of the anti-HuIFN- $\alpha$  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- $\alpha$  reference reagent against 10 LU/ml of HuIFN- $\alpha$ 2 as well as the HuIFN- $\alpha$  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 as the negative logarithm) should be reported with its standard deviation, along with the assigned titer of the anti-HuIFN- $\alpha$  Reference Reagent G037-501-572, 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.
4. Kawade, Y. Quantitation of neutralization of interferon by antibody. *Methods in Enzymology*. 119:558-573, 1986.
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 the proposed international reference human anti-HuIFN- $\alpha$  antiserum G037-501-572

HuIFN $\alpha$ antigen <sup>a/</sup>	Reciprocal of log <sub>10</sub> titers and variance within 13 laboratories obtained with the indicated interferon antigen (number of titrations)							log <sub>10</sub> GMT <sup>b/</sup>	GMT <sup>b/</sup>
	1	2	3	4	5	6	7		
Leukocyte	4.25 ± 0.30(11)	3.70 ± 0.29(11)	3.25 ± 0.19(4)	3.56 ± 0.16(14)	3.99 ± 0.23(5)	3.40 ± 0.14(4)	2.80 ± 0.18(3)		
Lympho- blastoid	4.37 ± 0.15(11)	3.89 ± 0.19(11)	3.66 ± 0.19(3)	3.56 ± 0.11(14)	3.87 ± 0.29(5)	3.64 ± 0.2(5)	3.18 ± 0.21(19)		
$\alpha$ 2a	4.42 ± 0.30(11)	4.50 ± 0.26(11)	3.74 ± 0.13(3)	3.95 ± 0.11(14)	4.06 ± 0.30(5)	3.99 ± 0.24(4)	3.66 ± 0.08(7)		
$\alpha$ 2b	4.63 ± 0.24(11)	4.34 ± 0.25(11)	3.86 ± 0.23(3)	3.99 ± 0.14(14)	4.17 ± 0.41(5)	4.03 ± 0.19(5)	3.46 ± 0.14(8)		
$\alpha$ 2c	4.69 ± 0.29(11)	3.93 ± 0.23(11)	3.89 ± 0.12(3)	4.10 ± 0.15(14)	3.58 ± 0.31(5)	3.91 ± 0.24(4)	3.43 ± 0.16(12)		
$\alpha$ 1	3.28 ± 0.36(11)	3.34 ± 0.17(11)	2.59 ± 0.26(3)	2.60 ± 0.12(9)	3.30 ± 0.28(4)	2.96 ± 0.14(5)	2.36 ± 0.25(11)		
$\alpha$ 1/8	4.44 ± 0.23(11)	3.71 ± 0.17(9)	--- --	3.90 ± 0.17(13)	3.46 ± 0.21(5)	3.92 ± 0.16(4)	3.29 ± 0.15(10)		
	8	9	10	11	12	13			
Leukocyte	3.66 ± 0.19(8)	3.27 ± 0.31(3)	3.88 ± 0.21(5)	3.09 ± 0.19(5)	4.11 ± 0.21(5)	2.38 ± 0.25(5)	3.50 ± 0.49 n=13 (83)	3000	
Lympho- blastoid	3.92 ± 0.18(5)	3.81 ± 1.03(3)	3.80 ± 0.16(5)	3.67 ± 0.10(5)	4.34 ± 0.17(5)	3.26 ± 0.27(5)	3.74 ± 0.39 n=13 (96)	5000	
$\alpha$ 2a	4.12 ± 0.16(7)	3.56 ± 0.07(3)	3.96 ± 0.15(5)	3.87 ± 0.11(5)	4.23 ± 0.17(5)	3.14 ± 0.14(5)	3.98 ± 0.36 n=13 (85)	9500	
$\alpha$ 2b	4.27 ± 0.25(8)	3.89 ± 0.29(3)	3.80 ± 0.13(4)	4.00 ± 0.10(5)	4.09 ± 0.12(5)	3.50 ± 0.27(5)	3.99 ± 0.34 n=13 (87)	9700	
$\alpha$ 2c	4.21 ± 0.13(8)	--- --	3.75 ± 0.16(4)	3.96 ± 0.10(5)	4.20 ± 0.16(5)	3.38 ± 0.21(5)	3.88 ± 0.38 n=12 (87)	7600	
$\alpha$ 1	3.13 ± 0.22(7)	--- --	2.78 ± 0.13(5)	2.84 ± 0.06(5)	--- --	2.42 ± 0.25(5)	2.86 ± 0.36 n=11 (76)	700	
$\alpha$ 1/8	4.10 ± 0.34(8)	--- --	3.70 ± 0.07(5)	3.86 ± 0.07(5)	3.72 ± 0.29(5)	3.32 ± 0.14(5)	3.77 ± 0.36 n=11 (80)	5000	

<sup>a/</sup> HuIFN- $\alpha$  antigens utilized: Leukocyte = International Reference Preparation (IRP) 69/19;

Lymphoblastoid = International Standard (IS) Ga23-901-532;

$\alpha$ 2a = IS Gxa01-901-535;

$\alpha$ 2b = IS 82/576;

$\alpha$ 1 = IS 83/514;

$\alpha$ 2c from Boehringer;

$\alpha$ 1/8 from Ciba-Geigy.

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