

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

#### Catalog No. NR-3091

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#### Lot (NIAID Catalog) No. G028-501-568

#### 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 to human fibroblast interferon beta

Host: Suffolk-Hampshire female yearling sheep

#### Immunizing Antigen:

Human fibroblast interferon prepared in diploid cell strains induced with poly (I) poly (C) and purified to a specific activity of  $1 \times 10^6$  units per mg protein

NIAID Class: Research Reference Reagent

Research Reference Reagent Note (attached): No. 24

Adjuvant used: Freund's complete in booster inoculations

#### Material Provided/Storage:

Composition: Lyophilized

Original Volume: 0.5 mL

Storage Temperature: 4°C or colder

Reconstitution: 0.5 mL sterile distilled water

#### Functional Activity:

Neutralizing Titer: 1:12,000 against 8 to 10 Laboratory Units of human interferon beta

#### Purity:

Sterility: No evidence of bacterial or fungal contamination

#### Producer and Contract:

Medical College of Pennsylvania N01-AI-82568

#### 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 Fibroblast Interferon Beta (antiserum, Sheep), NR-3091."

#### 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).

#### Disclaimers:

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#### References:

1. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. "Protein Measurement with the Folin Phenol Reagent." J. Biol. Chem. 193 (1951): 265-275. PubMed: 14907713.
2. Armstrong, J. A. "Semi-Micro, Dye-Binding Assay for Rabbit Interferon." Appl. Microbiol. 21 (1971): 723-725. PubMed: 4325022.
3. Havell, E. A. and J. Vilcek. "Production of High-Titered Interferon in Cultures of Human Diploid Cells." Antimicrob. Agents Chemother. 2 (1972): 476-484. PubMed: 4670440.
4. Mogensen, K. E., L. Pyhala, and K. Cantell. "Raising Antibodies to Human Leukocyte Interferon." Acta Pathol.

- Microbiol. Scand. [B] 83 (1975): 443–450. PubMed: 1180059.
5. Edy, V. G., et al. "Purification of Interferon by Adsorption Chromatography on Controlled Pore Glass." J. Gen. Virol. 33 (1976): 517–521. PubMed: 1036458.
  6. Billiau, A., et al. "Human Fibroblast Interferon for Clinical Trials: Production, Partial Purification, and Characterization." Antimicrob. Agents Chemother. 16 (1979): 49–55. PubMed: 475374.

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RESEARCH REFERENCE REAGENT NOTE # 24  
SHEEP ANTISERUM TO HUMAN FIBROBLAST INTERFERON  
CATALOG NUMBER G-028-501-568

Research Resources Branch  
National Institute of Allergy and Infectious Diseases  
National Institutes of Health  
Bethesda, Maryland 20205

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Preparation

Antibodies to human fibroblast interferon were produced in a Suffolk-Hampshire female yearling sheep based on the procedure of Mogensen, et al. (1). The sheep received twelve weekly injections of  $1.2 \times 10^7$  units per injection of human fibroblast interferon, prepared in diploid cell strains induced with poly I:C, and purified to a specific activity of  $1 \times 10^6$  units per mg protein (2). The interferon was obtained from the Rega Institute, Leuven, Belgium. Six weeks after the twelfth injection, a booster inoculation of  $2.5 \times 10^7$  units of the same human fibroblast interferon was admixed with Freund's Complete Adjuvant and injected into several intramuscular sites. Bleedings were begun seven days later. Other booster injections, followed by bleedings and six week rest periods were carried out until maximum antibody titers were achieved. Sera of maximum titer obtained from this animal, were used for preparing this reference standard.

Greater than 95 percent of the antibodies to known contaminants present in the interferon preparation used for immunization were removed by immunoabsorption techniques utilizing antigens bound to Sepharose 4B. The antigens were those components of an interferon preparation which did not bind to a Controlled Pore Glass column (3), supplemented with bovine albumin, human plasma protein and soluble extract prepared from diploid human fibroblasts. The globulin portion of the serum was separated by precipitation with 50 percent ammonium sulfate, dialyzed versus 0.01 M sodium phosphate buffer pH 7, then sterilized by filtration. The globulin was dispensed (0.5 ml per ampule), freeze-dried and sealed by The American Type Culture Collection.

Recommendations for Reconstitution

Add 0.5 ml of sterile physiologic saline solution or an appropriate medium to the lyophilized powder. Precautions should be taken to avoid loss of material in the neck or stem of the ampule. The reconstituted globulin can be diluted and stored indefinitely at  $-20^{\circ}\text{C}$  or lower.

Interferon Neutralization Assay

The assay procedure used at The Medical College of Pennsylvania is similar to the interferon assay in microtiter plates (4,5), except that 50  $\mu\text{l}$  volumes of serial two-fold dilutions of antiserum are preincubated for 1 hr at  $37^{\circ}\text{C}$  with 50  $\mu\text{l}$  of graded interferon dilutions covering the range from 1-32 units before addition of the 30,000 human FS-4 cells per well. Encephalomyocarditis virus at a multiplicity of 0.2 was used for challenge. Interferon, virus and cell controls are included in each test. The antiserum is titrated against several dilutions of test antigens in order to select, for computation of the titer, the series with the appropriate number of interferon units available for neutralization by antibody. The highest dilution which neutralized 8-10 reference units of interferon by partially restoring viral cytopathic effect, corrected for 1 ml volume, represented the titer of the antiserum. The human fibroblast interferon reference standard used was G-023-902-527.

### Reagent Control

A control globulin preparation for this antibody to human fibroblast interferon is also available (G-029-501-568).

Prepared by: Kurt Paucker, Barbara Dalton and Clifton A. Ogburn  
The Medical College of Pennsylvania  
Philadelphia, Pennsylvania 19129  
Contract No. 1 AI 82568

### References

1. Mogensen, K.E., Pyhälä, L. and Cantell, K. (1975) Acta Path. Microbiol. Scand., Sect. B 83:443-450.
2. Billiau, A., Van Damme, J., Van Leuven, F., Edy, V.G., DeLey, M., Cassiman, J., Van Den Berghe, H. and DeSomer, P. (1979) Antimicrob. Agents and Chemother. 16:49-55.
3. Edy, V.G., Braude, I.A., DeClercq, E., Billiau, A. and DeSomer, P. (1976) J. Gen. Virol. 33:517-521.
4. Havell, E.A. and Vilček, J. (1972) Antimicrob. Agents and Chemother. 2: 476-484.
5. Armstrong, J.A. (1971) Appl. Microbiol. 21:723-725.
6. Lowry, O.H., Rosebrough, J., Farr, L. and Randall, R.J. (1951) J. Biol. Chem. 193:265-275.