Product Information Sheet for NR-3074

Rabbit Interferon

Catalog No. NR-3074

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Lot (NIAID Catalog) No. G019-902-528

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: Rabbit interferon
NIAID Class: WHO International Standard
Research Reference Reagent Note (attached): No. 10A
Titer: 10,000 International Units/ampoule
Molecular Weight (G-200 Sephadex): 45,000 daltons

Method of Preparation:

Tissue Culture System: Rabbit kidney cells [primary and secondary (through 3rd passage)] infected with bluetongue virus (strain BT-8)
Medium: Eagle's Minimum Essential Medium + 2% fetal bovine serum
Treatment: Partially purified on zeolite, pH 3.5 for 48 hours. Suspended in 0.1 M sodium phosphate, pH 7 with 0.5% bovine plasma albumin
Freeze-drying: Residual moisture 3%; back-filled with argon and heat-sealed

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: 50% of activity remaining after 18 hours at 90°C
Ultracentrifugation: No loss in activity

Purity:

Activity on Heterologous Cells: None (< 10 Laboratory Units/mL) on mouse L cells by GDVII HA-yield reduction test; 20 Laboratory Units/mL on human BUD-8 cell strain
Sterility: No evidence of bacterial, mycoplasmal, viral 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: Rabbit Interferon, NR-3074."

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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References:

1. Jameson, P., M. A. Dixon, and S. E. Grossberg. "A Sensitive Interferon Assay for Many Species of Cells: Encephalomyocarditis Virus Hemagglutinin Yield Reduction." Proc. Soc. Exp. Biol. Med. 155 (1977): 173–178. PubMed: 194253.

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2. Jameson, P., C. Schoenherr, and S. E. Grossberg. "Bluetongue Virus, an Exceptionally Potent Interferon Inducer in Mice." Infect. Immun. 20 (1978): 321–323. PubMed: 208975.

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RESEARCH REFERENCE REAGENTS NOTE #10A
Freeze-Dried Rabbit Reference Interferon
Catalog Number G-019-902-528

WHO INTERNATIONAL STANDARD*

RESEARCH RESOURCES BRANCH
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, Maryland 20205

December 1976

(Revised June 1980)

*Journal of Biological Standardization
(1979), 7:383-395

Research Reference Reagents Note #10A
Freeze-dried Rabbit Reference Interferon (G-019-902-528)

Preparation: Interferon was prepared by the inoculation of rabbit kidney cell cultures (primary through third passage) with an attenuated derivative of the BT-8 strain of bluetongue virus. Cultures were incubated in Eagle's minimun essential medium supplemented with 2% fetal bovine serum, and the supernatant fluids were collected 24 hours after virus inoculation. Viral infectivity was destroyed by dialysis at pH 3.5 for 48 hours. Interferon was partially purified 3-fold (with about 25% recovery of initial activity) by the method of Sonnabend and Katsoyannis (NIH contract 70-2129) employing adsorption to and elution from zeolite. Crystalline bovine plasma albumin was added to a final concentration of 0.5% and the interferon preparation finally dialyzed against 0.1 M sodium phosphate buffer pH 7.0. After sterilization by filtration, interferon titer and sterility were determined. The material was then dispensed as 1.0 ml aliquots into borosilicate ampoules, freeze-dried under vacuum to a residual moisture of 3% and an atmosphere of argon was added. The ampoules were sealed by heat and the seal coated with neoprene. The last sample from each group of 38 ampoules was tested for bacterial and fungal contamination. Ampoules are stored at -70 C and can be shipped at ambient temperatures.

Recommendations for Reconstitution: 1.0 ml of sterile distilled water should be added to the lyophilized powder, care being taken to avoid loss of any material in the neck or stem of the ampoule. Portions of this reconstituted material can be stored at -70 C, or an appropriate (e.g., 1:10) dilution can be made preferably in 0.1 M sodium phosphate buffer pH 7 containing 0.5% crystalline bovine plasma albumin (BPA); Hanks' salt solution with 0.5% crystalline BPA or serum-containing culture medium may be substituted. For optimum, long-term preservation of stability, storage of samples of the liquid material should be at -70 C.

Assay: The preparation has been assayed at The Medical College of Wisconsin, Milwaukee, by the encephalomyocarditis virus hemagglutinin yield-reduction (YR) method developed in that laboratory and by the vesicular stomatitis virus (VSV) plaque-reduction (PR) test, using the RK-13 cell line (obtained from Flow Laboratories). Methods of bioassay in several other laboratories employed VSV and included: PR with the RK-13 cell line, reduction in cytopathogenic effect by visual evaluation with the RK-13 cell line or by dye-uptake with secondary rabbit kidney cells. No residual virus was detected after three blind passages in BHK-21 cells. Sterility was tested before freeze-drying by inoculation of mycoplasma medium, brain heart infusion agar (BHI) and Sabouraud's agar (SA). Mycoplasma tests were observed after two weeks at 36 C in a nitrogen atmosphere, which supported growth of a known control mycoplasma culture. Twenty-eight samples taken from the lot after freeze-drying were screened for sterility in SA and BHI agar. Cultures were held for two weeks (36 C for BHI, 22 C for SA).

Tests Results: The preparation contains no detectable bluetongue virus, mycoplasma, bacteria or fungi. The interferon product was characterized prior to freeze-drying as being nonsedimentable, stable at pH 2 for 48 hours, and inactivated by trypsin; 75% was inactivated after 1 hour at 56 C. It is estimated to have a molecular weight of 45,000 daltons by G-200 Sephadex chromatography.

Potency was determined from data contributed by seven laboratories (Table 1).

Table 1. Titer of freeze-dried rabbit interferon standard G-019-902-528 compared with frozen rabbit serum standard G-019-902-028 by three different types of assay methods.

Type of assay	N	G019-901-028	G019-902-528	Significant Difference	Ratio ($\log_{10}$) G019-901-028 / G019-902-528
RK-13, VSV-PR	18	3.89	3.53	Yes ($p < .001$)	0.36
2^{o}RK, VSV-CPE (dye)	8	4.48	3.92	Yes ($p < .001$)	0.56
RK-13, VSV-CPE	9	3.02	3.07	No	-0.05

The original frozen interferon standard had a titer of 20,000 ($10^{4.3}$) units, and the new standard is about one-half the potency ($\log_{10}$ ratio = 0.3). Its assigned titer relative to the previous reference reagent is therefore 10,000 units.

Use of Reference Interferon: The purpose of the reference interferon is to provide a comparison of the sensitivities of interferon assays among different laboratories. Each laboratory should assay the reference interferon in comparison with their own laboratory interferon standard. The observed titer of the reference preparation of rabbit interferon should be stated in each publication. It is recommended that several titrations of the standard be done so that the mean interferon titer observed may be stated along with the range or standard deviation. The mean titer which might be obtained, for example, by four titrations could fall between 1,673 and 6,864 units with the plaque-reduction method. Larger numbers of titrations will improve the estimate by reducing the possible range (Table 2).

Table 2. Range of expected mean titers for the indicated number of titrations of G019-902-528 by the VSV plaque-reduction (PR) method.*

Extremes of range	Titers expected for the indicated number of titrations			
	3	5	10	18
low	1500	1803	2169	2430
high	7656	6371	5296	4727

*The projected values given are based on the observed (unadjusted) mean titer obtained by the VSV-PR method for this standard.

Stability: The freeze-dried material has shown no loss in titer in the linear non-isothermal accelerated storage test (progressive increase in temperature from 50 C to 90 C over 28 hours). Fifty per cent of the activity remained after heating at 90 C continuously for 18 hours. Current estimates predict unlimited stability at -70 C; predicted stability at other temperatures is based on multiple isothermal accelerated storage tests at 52, 60 and 68 C. The time predicted to lose 1000 units of activity is about 55 days at 37 C, 360 days at 20 C, and 6.3 years at 4 C.

Product Information Summary

I.	Reagent	
	A. Proper Name	Rabbit Interferon (freeze-dried)
	B. Catalog Number	G-019-902-528
	C. Class	Research Reference Reagent
II.	Characteristics	
	A. Method of Preparation	
	1. TC System and/or animal used	Rabbit kidney cells [primary and secondary (through 3rd passage)] infected with bluetongue virus (strain BT-8)
	2. Medium use	MEM + 2% fetal bovine serum
	3. Treatment	pH 3.5 for 48 hours and zeolite elution
	4. Partial purification	Zeolite treatment yielding 3-fold purification. Final suspension in 0.1 M sodium phosphate pH 7 with 0.5% crystalline bovine plasma albumin (Armour)
	5. Freeze-drying	Residual moisture 3%, in argon atmosphere in sealed borosilicate ampoules
III.	Potency (Test Method Used)	
	A. Yield-reduction assay with single growth cycle of EMC virus	In RK-13 cell line, $10^{3.87 \pm 0.11}$ units (0.11 = S.D.; N = 10)
	B. Molecular weight (G-200 Sephadex)	45,000
	C. Ultracentrifugation (100,000 g for 2 hrs)	No loss in activity
	D. Stability (prior to freeze-drying)	100% activity remaining after 48 hrs at pH 3.5 and 75% activity after an additional 48 hrs at pH 2; about 25% activity remaining after 60 min at 56°C
	E. Trypsinization	Activity destroyed

IV. Purity (Test Method Used)

A. Activity on heterologous cells	None (<10) on mouse L cells by GDVII HA-yield reduction test; $10^{1.3}$ units on human BUD-8 cell strain by EMC HA-yield reduction
B. Bacterial sterility (brain heart infusion agar)	Sterile (after 2 weeks at 36°C)
C. Fungal sterility (Saboraud's agar)	Sterile (after 3 weeks at 22°C)
D. Mycoplasma	Sterile (after 2 weeks in N_2 at 36°C)
E. Viral	No bluetongue virus detected after blind passage in BHK-21 cells

V. Ampouled Preparation

A. Contents	Rabbit interferon, freeze-dried
B. Volume	1 ml before freeze-drying
C. Date of last potency test	April 1976
D. Storage temperature	-70°C recommended
E. Stability after freeze-drying	50% of activity remaining after 18 hrs at 90°C

VI. Producer

S. E. Grossberg, M.D.; P. Jameson, Ph.D.; and D. Greiff, D.Sc.
The Medical College of Wisconsin

VII. References

A. Bluetongue virus induction of interferon and antiviral activity, Patricia Jameson, Christine Schoenherr, and Sidney E. Grossberg, submitted for publication

B. A sensitive interferon assay for many species of cells: encephalomyocarditis virus hemagglutinin yield-reduction, Patricia Jameson, Mary A. Dixon, and Sidney E. Grossberg, submitted for publication