Giardia lamblia, Strain Be-1

Catalog No. NR-9237

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Contributor:

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Product Description:

Protozoa Classification: *Hexamitidae, Giardiinae, Giardia*
Species: *Giardia lamblia* (also referred to as *Giardia intestinalis* and *Giardia duodenalis*)
Strain: Be-1 (Beaver-1, IP-0482:1)
Original Source: *Giardia lamblia* (*G. lamblia*), strain Be-1 was isolated by Dr. Faubert and M. Belosevic from cysts obtained from beaver feces found in pond mud in Banff National Park, Canada. The cysts were axenized at the NIH.[1]
Comments: The whole genome shotgun sequencing project of *Giardia lamblia*, strain WB, clone C6 is in progress (GenBank: AACB00000000).[2,3]

G. lamblia is a pear-shaped, flagellated protozoan that causes a wide variety of gastrointestinal complaints and is one of the most common causes of parasite infection of humans worldwide, and the second most common in the United States. The disease is commonly water-borne because *Giardia* cysts are resistant to the chlorine levels in normal tap water and survive well in cold mountain streams. Food-borne transmission is rare but can occur with ingestion of raw or undercooked foods. Giardiasis is a zoonosis, and cross-infectivity among beaver, cattle, dogs, rodents, and bighorn sheep provides a constant reservoir.[4] The life cycle of *Giardia* consists of two stages: the fecal-orally transmitted cyst and the disease-causing trophozoite. Cysts are passed in a host's feces, remaining viable in a moist environment for months. Ingestion of 10 to 25 cysts can cause infection in humans.[5]

Material Provided:

Each vial of NR-9237 contains approximately 0.5 mL of culture in cryopreservative. Please see Appendix I below for cryopreservation instructions.

Packaging/Storage:

NR-9237 was packaged aseptically in screw-capped plastic cryovials and is provided frozen on dry ice. The product should be stored at cryogenic temperature (-130°C or colder), preferably in the vapor phase of a liquid nitrogen freezer. If liquid nitrogen storage facilities are not available, frozen cryovials may be stored at -70°C or colder for approximately one week. Note: Do not under any circumstances store vials at temperatures warmer than -70°C. Storage under these conditions will result in the death of the culture.

To insure the highest level of viability, the culture should be initiated immediately upon receipt. Any warming of the product during shipping and transfer must be avoided, as this will adversely affect the viability of the product. For transfer between freezers and for shipping, the product may be placed on dry ice for brief periods, although use of a portable liquid nitrogen carrier is preferred. Please read the following recommendations prior to using this material.

Growth Conditions:

Growth Media:
ATCC® medium 2695 (previously ATCC® medium 1404), supplemented with Diamond's Vitamin Solution and 10% heat-inactivated adult bovine serum. Please see Appendix II for media preparation instructions.
Note: An alternative to ATCC® medium 2695 is ATCC® medium PRA-2155.
Incubation:
Temperature: 35°C
Atmosphere: axenic and microaerophilic
Propagation:

1. Thaw frozen ampoule in a 35°C water bath, for 2 to 3 min. Immerse the ampoule just sufficient to cover the frozen material. Do not agitate the ampoule.
2. Immediately after thawing, aseptically transfer contents to a 16 x 125 mm screw-capped borosilicate glass test tube containing 13 mL of ATCC® medium 2695. Incubate the tube on a 15° horizontal slant at 35°C.

Maintenance:

1. When the culture has reached or is near peak density, place the tubes on ice for 10 minutes.
2. Gently invert the culture tube 10 times and aseptically transfer a 0.1 to 0.4 mL aliquot to a screw-capped test tube containing 13 mL ATCC® medium 2695.
3. Incubate the culture on a 15° horizontal slant at 35°C.
4. Transfer the culture every 3 to 4 days as described in Maintenance steps 1 and 2. The transfer interval will depend on the size of the inoculum and the quality of the medium. This should be determined empirically by examining the culture on a daily basis until conditions for stable growth have been achieved. Do not allow the culture to overgrow. Viability of the culture may be affected soon after reaching peak density.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: *Giardia lamblia*, Strain Be-1, NR-9237."

Biosafety Level: 2

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. Nash, T. E., et al. "Restriction-Endonuclease Analysis of DNA from 15 *Giardia* Isolates Obtained from Humans and Animals." J. Infect. Dis. 152 (1985): 64-73. PubMed: 2409186.
2. Morrison, H. G., et al. "Genomic Minimalism in the Early Diverging Intestinal Parasite *Giardia lamblia*." Science 317 (2007): 1921-1926. PubMed: 17901334.
3. McArthur, A. G., et al. "The *Giardia* Genome Project Database." FEMS Microbiol. Lett. 189 (2000): 271-273. PubMed: 10930750.
4. Wallis, P. M., et al. "Reservoirs of *Giardia* Spp. in Southwestern Alberta." J. Wildl. Dis. 20 (1984): 279-283. PubMed: 6397598.
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6. Nash, T. E. and D. B. Keister. "Differences in Excretory-Secretory Products and Surface Antigens among 19 Isolates of *Giardia*." J. Infect. Dis. 152 (1985): 1166-1171. PubMed: 4067331.
7. Belosevic, M., et al. "*Giardia lamblia* Infections in Mongolian Gerbils: An Animal Model." J. Infect. Dis. 147 (1983): 222-226. PubMed: 6827139.

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APPENDIX I: CRYOPRESERVATION

1. Harvest cells from a culture that is at or near peak density. To detach cells from the wall of the culture tubes place on ice for 10 min. Invert tubes several times until the majority of the cells are in suspension. Centrifuge tubes at 800 x g for 5 minutes.
2. Adjust the concentration of cells to 1-2 x 10^7/mL in fresh medium.
3. Before centrifuging prepare a 24% (v/v) solution of sterile DMSO in fresh medium containing 8% (w/v) sucrose. The solution is prepared as follows:
    a) Add 1.05 g sucrose to 10 mL of fresh medium and filter sterilize through a 0.2 µm filter
    b) Add 2.4 mL of DMSO to an ice cold 20 x 150 mm screw-capped test tube
    c) Place the tube on ice and allow the DMSO to solidify (~ 5 min) and then add 7.6 mL of ice cold medium prepared in step 3a. The final concentration will be 24% (v/v) DMSO and 8% (w/v) sucrose.
    d) Invert several times to dissolve the DMSO.
    e) Allow to warm to room temperature.
4. Mix the cell preparation and the cryoprotective agent, prepared in step 3, in equal portions. Thus, the final concentration will equal 12% (v/v) DMSO, 4% (w/v) sucrose and 10^7 cells/mL. The time from the mixing of the cell preparation and DMSO stock solution before the freezing process is begun should be no less than 15 min. and no longer than 30 min.
5. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryovials.
6. Place the vials in a controlled rate freezing unit. From room temperature, cool at -1°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through the heat of fusion. At -40°C, plunge into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing apparatus. Place the apparatus at -80°C for 1.5 to 2 hours and then plunge ampoules into liquid nitrogen (the cooling rate in this apparatus is approximately -1°C/min).
7. The frozen preparations should be stored in either the vapor or liquid phase of a nitrogen freezer. Stability of frozen preparations is higher at -130°C as compared to temperatures warmer than -130°C.

APPENDIX II: MEDIA

ATCC® Medium 2695 (Keister's Modified TYI-S-33)

Casein Digest (BD Trypticase 211705)	20.0 g
Yeast Extract (BD 212750)	10.0 g
Bovine Bile (Sigma B-8381)	0.75 g
NaCl	2.0 g
L-Cysteine HCl	2.0 g
Ascorbic Acid	0.2 g
K_2HPO_4	1.0 g
KH_2PO_4	0.6 g
Ferric Ammonium Citrate	22.8 mg
Distilled water	880 mL

1. Dissolve the ingredients in 880 mL of distilled water in the order indicated.
2. Adjust the pH to 7.0 to 7.2 with 1 N NaOH.
3. Filter sterilize.
4. To prepare the complete medium, aseptically add 20 mL of Diamond's Vitamin Solution and 100 mL of heat-inactivated adult bovine serum.
5. Mix thoroughly and distribute 13 mL aliquots into 16 x 125 mm screw-capped borosilicate glass test tubes. Store at 4°C to 8°C in the dark. Use within 7 to10 days. Long term storage may result in the formation of precipitates and failure to support growth of *Giardia*.

NOTE: Serum is heat-inactivated by exposure to 56°C for 30 minutes to inactivate proteins of the complement pathway.

Diamond's Vitamin Solution

Solution 1: (DL-6,8-Thioctic acid [DL-α-Lipoic acid], 1 mg/mL).
Dissolve 100 mg of DL-6,8-Thioctic acid (oxidized form, Sigma T1395) in 100 mL of absolute ethanol.
Solution 2: (Vitamin B_{12}, 0.4 mg/mL).
Dissolve 40 mg of vitamin B12 (Sigma V2876) in 100 mL distilled water.
Solution 3: (Tween 80, 50% w/v).
Dissolve 50 g of Tween 80 (Sigma P1754) in 100 mL absolute ethanol.
Solution 4 (All components from Sigma):

α-tocopherol phosphate, disodium salt	0.025 mg
d-biotin	0.025 mg
Calciferol (Vitamin D2)	0.250 mg
Calcium D-(+)pantothenate	0.025 mg
Choline chloride	1.250 mg
Folic acid	0.025 mg
i-Inositol	0.125 mg
Menadione (Vitamin K3)	0.025 mg
Niacin	0.0625 mg
Niacinamide	0.0625 mg
p-aminobenzoic acid	0.125 mg
Pyridoxal HCl	0.0625 mg
Pyridoxine HCl	0.0625 mg
Riboflavin	0.0625 mg
Thiamine HCl	0.025 mg
Vitamin A	0.250 mg
Distilled water	1 L

To prepare Diamond's Vitamin Solution combine the following:

Solution 1	0.4 mL
Solution 2	1.2 mL
Solution 3	0.4 mL
Solution 4	100.0 mL
Sterile distilled water	18.0 mL

Filter sterilize. Store at -20°C.