

***Giardia lamblia*, Strain WB, Clone 6 (axenic)**

Catalog No. NR-9706

(Derived from ATCC® 50803™)

For research use only. Not for use in humans.

Contributor:

ATCC®

Manufacturer:

BEI Resources

Product Description:

Protozoa Classification: Hexamitidae, Giardiinae, *Giardia*

Species: *Giardia lamblia* (also referred to as *Giardia intestinalis* and *Giardia duodenalis*)¹

Strain: WB, clone 6

Source: *Giardia lamblia* (*G. lamblia*), strain WB, clone C6 was produced for investigations of variable surface glycoprotein expression.^{2,3} *G. lamblia*, strain WB was isolated from a 29-year-old male in Afghanistan.^{4,5}

Comments: The whole genome shotgun sequencing project of *G. lamblia*, strain WB, clone C6 has been sequenced (GenBank: [AACB00000000](https://www.ncbi.nlm.nih.gov/nuclseq/AACB00000000)).^{6,7}

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 in 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 the 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.⁸ 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.⁹

Material Provided:

Each vial of NR-9706 contains approximately 0.5 mL of cells in cryopreservative [12% dimethylsulfoxide (DMSO)]. Please refer to Appendix I for cryopreservation instructions.

Packaging/Storage:

NR-9706 was packaged aseptically in screw-capped plastic cryovials and is provided frozen on dry ice. The product should be stored at -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 ensure 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:

Keister's Modified Trypticase-Yeast Extract-Iron-Serum (TYI-S-33) medium supplemented with Diamond's Vitamin solution and 10% heat-inactivated adult bovine serum or equivalent (Appendix II)

Incubation:

Temperature: 35°C

Atmosphere: Microaerophilic

Propagation:

1. To establish a culture from the frozen state, place a vial in a 35°C water bath for 2 to 3 minutes, until thawed. Immerse the vial just enough to cover the frozen material. Do not agitate the vial.
2. Immediately after thawing, transfer the vial contents to a 16 × 125 mm screw-capped borosilicate glass test tube containing 13 mL of growth medium.
3. Screw the cap on tightly and incubate at a 15° horizontal slant at 35°C. Observe the culture daily and subculture when peak density is observed.

Maintenance:

1. When the culture is at or near peak density, ice the culture for 10 minutes.
2. Gently invert the tube 10 times and aseptically transfer a 0.1 mL to 0.4 mL aliquot to screw-capped test tubes containing 13 mL of freshly prepared growth media.
3. Incubate at 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.

Please refer to Appendix I for cryopreservation instructions.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Giardia lamblia*, Strain WB, Clone 6 (axenic), NR-9706."

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. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmb15/index.htm.

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References:

1. Adam, R. D. "Biology of *Giardia lamblia*." Clin. Microbiol. Rev. 14 (2001): 447-475. PubMed: 11432808.
2. Smith, P. D., et al. "Antigenic Analysis of *Giardia lamblia* from Afghanistan, Puerto Rico, Ecuador and Oregon." Infect. Immun. 36 (1982): 714-719. PubMed: 7085076.
3. Smith, P. D., et al. "Chronic Giardiasis: Studies on Drug Sensitivity, Toxin Production, and Host Immune Response." Gastroenterology 83 (1982): 797-803. PubMed: 7106510.
4. Gillin, F. D., et al. "Isolation and Expression of the Gene for a Major Surface Protein of *Giardia lamblia*." Proc. Natl. Acad. Sci. USA 87 (1990): 4463-4467. PubMed: 2352929.
5. Meng, T. C., M. L. Hetsko and F. D. Gillin. "Antigenic Switching of TSA 417, a Trophozoite Variable Surface Protein, following Completion of the Life Cycle of *Giardia lamblia*." Infect. Immun. 61 (1993): 5394-5397. PubMed: 8225614.
6. Morrison, H. G., et al. "Genomic Minimalism in the Early Diverging Intestinal Parasite *Giardia lamblia*." Science 317 (2007): 1921-1926. PubMed: 17901334.

7. McArthur, A. G., et al. "The *Giardia* Genome Project Database." FEMS Microbiol. Lett. 189 (2000): 271-273. PubMed: 10930750.
8. Wallis, P. M., et al. "Reservoirs of *Giardia* spp. in Southwestern Alberta." J. Wildl. Dis. 20 (1984): 279-283. PubMed: 6397598.
9. Kucik, C. J., G. L. Martin and B. V. Sorter. "Common Intestinal Parasites." Am. Fam. Physician 69 (2004): 1161-1168. PubMed: 15023017.

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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 minutes. Invert tubes several times until the majority of the cells are in suspension. Centrifuge tubes at $800 \times g$ for 5 minutes.
2. Adjust the cell concentration to 1×10^7 to 2×10^7 cells per milliliter with fresh medium.
3. Before centrifuging, prepare a 24% (v/v) solution of sterile dimethylsulfoxide (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 sterile filter through a $0.2 \mu\text{m}$ filter.
 - b) Add 2.4 mL of DMSO to an ice-cold 20×150 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 per milliliter.

Note: 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 minutes and no longer than 30 minutes.
5. Dispense 0.5 mL aliquots into 1 mL to 2 mL sterile plastic screw-capped vials for cryopreservation.
6. Place the vials in a controlled rate freezing unit. From room temperature, cool the vials at -1°C per minute to -40°C . If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C per minute through this phase. At -40°C , plunge vials into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing container. Place the container at -80°C for 1.5 to 2 hours and then plunge vials into liquid nitrogen.
7. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).

APPENDIX II: KEISTER'S MODIFIED TYI-S-SS MEDIUM

1. Prepare the Keister's Modified TYI-S-SS medium (see formulation below) by dissolving the components in 880 mL of distilled water in the order indicated, adjust the pH to 7.0 to 7.2 with 1 N NaOH, and sterile filter.

Keister's Modified TYI-S-33 Medium

Casein Digest (BD Trypticase 211705)	20.0 g
Yeast Extract (BD 212750)	10.0 g
Bovine Bile (Sigma B8381)	0.75 g
NaCl	2.0 g
L-Cysteine HCl	2.0 g
K ₂ HPO ₄	1.0 g
KH ₂ PO ₄	0.6 g
Ferric Ammonium Citrate	22.8 mg
Distilled water	880 mL

2. Prepare each of the four individual Diamond's Vitamin Stock Solutions (listed below) and sterile filter each one using a 0.22 µm filter.

Diamond's Vitamin Stock Solution 1: (DL-6,8-Thioctic acid [DL-α-Lipoic acid], 1 milligrams per milliliter)

Dissolve 100 mg of DL-6,8-Thioctic acid (oxidized form, Sigma T1395) in 100 mL of absolute ethanol.

Diamond's Vitamin Stock Solution 2: (Vitamin B₁₂, 0.4 milligrams per milliliter)

Dissolve 40 mg of vitamin B12 (Sigma V2876) in 100 mL distilled water.

Diamond's Vitamin Stock Solution 3: (Tween 80, 50% w/v)

Dissolve 50 g of Tween 80 (Sigma P1754) in 100 mL absolute ethanol.

Diamond's Vitamin Stock Solution 4:

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

3. Prepare Diamond's Vitamin Solution by combining the stock solutions as follows:

Diamond's Vitamin Stock Solution 1	0.4 mL
Diamond's Vitamin Stock Solution 2	1.2 mL
Diamond's Vitamin Stock Solution 3	0.4 mL
Diamond's Vitamin Stock Solution 4	100.0 mL
Sterile, distilled water	18.0 mL

4. Aseptically prepare the complete growth medium by adding 20 mL of Diamond's Vitamin Solution and 100 mL of heat-inactivated adult bovine serum to the Keister's Modified TYI-S-SS medium and mix thoroughly.
5. Distribute 13 mL aliquots into 16 × 125 mm screw-capped borosilicate glass test tubes. Store at 4°C to 8°C in the dark. Use within 7 to 10 days. Long-term storage may result in the formation of precipitates and failure to support the growth of *Giardia*.

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