Product Information Sheet for NR-176

Entamoeba histolytica HB-301:NIH

Catalog No. NR-176
(Derived from ATCC® 30190™)

For research use only. Not for human use.

Contributor:
ATCC®

Product Description:
Protozoa Classification: *Entamoebidae*, *Entamoeba*
Agent: *Entamoeba histolytica*
Strain: HB-301:NIH
Original Source:[1] Feces from adult human male with amoebic dysentery from Burma (1960)
Comments: *Entamoeba histolytica* HB-301:NIH was deposited at ATCC® in 1972 by Dr. Louis S. Diamond[2,3], Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland. This isolate contains a polyhedral virus.[4,5]

Entamoeba histolytica is a pathogenic protozoan parasite that predominantly infects humans and other primates. The active (trophozoite) stage exists only in the host and in fresh feces. Cysts, the environmental survival form, live outside the host in water and soils and on foods. When swallowed they cause infections by excysting (to the trophozoite stage) in the digestive tract. *Entamoeba histolytica* results in an asymptomatic carrier state in most individuals, but can cause diseases ranging from chronic, mild diarrhea to fulminant dysentery.

Material Provided:
Each vial of NR-176 contains approximately 0.5 mL of culture in cryopreservative. Please see Appendix I below for cryopreservation instructions.

Packaging/Storage:
NR-176 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 2154: or equivalent
Incubation:
Temperature: 35–37°C
Atmosphere: Axenic and 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. Transfer the vial contents to a 16 x 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 trophozoite density is observed.
4. To subculture, ice the culture for 10 minutes and gently invert 20 times.
5. Aseptically transfer a 0.1 and 0.25 mL aliquot to freshly prepared 16 x 125 mm screw-capped borosilicate glass test tubes containing 13 mL of growth medium.
6. Repeat Step 3.

Please see Appendix I below for cryopreservation instructions.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: *Entamoeba histolytica* HB-301:NIH, NR-176."

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. 4th ed. Washington, DC: U.S. Government Printing Office, 2007. HHS Publication No. (CDC) 93-8395. This text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

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Manassas, VA 20108-4137 USA
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References:

1. Diamond, L. S. "Techniques of Axenic Cultivation of *Entamoeba histolytica* Schaudinn, 1903 and *E. histolytica*-Like Amebae." J. Parasitol. 54 (1968): 1047–1056. PubMed: 4319346.
2. Diamond, L. S. "Axenic Cultivation of *Entamoeba histolytica*." Science 134 (1961): 336–337. PubMed: 13722605.
3. Clark, C. G. and L. S. Diamond. "Methods for Cultivation of Luminal Parasitic Protists of Clinical Importance." Clin. Microbiol. Rev. 15 (2002): 329–341. PubMed: 12097242.
4. Diamond, L. S., C. F. T. Mattern, and I. L. Bartgis. "Viruses of *Entamoeba histolytica* I. Identification of Transmissible Virus-Like Agents." J. Virol. 9 (1972): 326–341. PubMed: 4335522.
5. Mattern, C. F. T., L. S. Diamond, and W. A. Daniel. "Viruses of *Entamoeba histolytica* II. Morphogenesis of the Polyhedral Particle (ABRM→HK-9)→HB-301 and the Filamentous Agent $(ABRM)_2$→HK-9." J. Virol. 9 (1972): 342–358. PubMed: 4335523.
6. Loftus, B., et al. "The Genome of the Protist Parasite *Entamoeba histolytica*." Nature 433 (2005): 865–868. PubMed: 15729342.

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APPENDIX I: CRYOPRESERVATION

1. Prepare CPMB-2 Basal Solution (see recipe below).
2. Prepare L-Cysteine/Ascorbic Acid Solution (see recipe below).
3. Harvest cells from several cultures that are in peak density of growth and place on ice for 10 minutes.
4. Gently invert tubes 20 times and centrifuge at 200 x g for 5 minutes.
5. While cells are centrifuging, prepare the Cryoprotective Solution:
   a) Place 1.0 mL of DMSO in a 16 x 125 mm screw-capped test tube and ice until solidified.
   b) Add 0.8 mL of the 2.5 M sucrose, remove from ice and invert until the DMSO is liquefied and return to ice bath.
   c) Add 0.2 mL of the L-Cysteine/Ascorbic Acid Solution to the mixture and mix.
   d) Add 6.0 mL of the CPMB-2 Basal Solution and mix.
   e) Add 2.0 mL heat inactivated bovine serum and mix.
6. Resuspend the cell pellets and pool to a final volume of approximately 10 mL with the supernatant.
7. Determine the cell density using a hemocytometer, and adjust the concentration between 5 x 10^5 and 1 x 10^6 cells/mL using fresh media. If the cell concentration is lower than 5 x 10^5 cells/mL, centrifuge the cell suspension, remove the supernatant, and resuspend the pellet in a volume that will yield a concentration between 5 x 10^5 and 1 x 10^6 cells/mL.
8. After the cell concentration is adjusted, centrifuge at 200 x g for 5 minutes.
9. Remove as much supernatant as possible and determine the volume removed.
10. Resuspend the cell pellet with a volume of the Cryoprotective Solution equal to the volume of the supernatant removed. Gently invert the tube several times to obtain a uniform cell density.
11. Dispense 0.5 mL aliquots into plastic sterile cryovials.
12. Place the vials in a controlled rate freezing unit. From room temperature, cool at -10°C/min until the liquid begins to freeze; from this point until -40°C is reached, cool at -1°C/min. At -40°C plunge the vials into liquid nitrogen. The cooling cycle should be initiated 15 to 30 minutes after the addition of DMSO to the cell preparation.
13. Store ampoules in a liquid nitrogen refrigerator until needed (-130°C or colder).

CPMB-2 Basal Solution

Yeast Extract	60.0 g
K_2HPO_4	1.0 g
KH_2PO_4	0.6 g
NaCl	2.0 g
Distilled water	1.0 L

Add the ingredients in the order listed above to the distilled water, mix and adjust the pH to 6.8. The solution should be autoclaved for 20 minutes at 121°C.

L-Cysteine/Ascorbic Acid Solution

L-Cysteine-HCl	1.0 g
Ascorbic Acid	0.1 g
10N NaOH	~ 0.7 mL
Distilled water	

Add 9.0 mL of distilled water to a 20 mL beaker and dissolve the first two components. While stirring, adjust the pH to 7.2 with 10 N NaOH (approximately 0.7 mL). Adjust the final volume to 10 mL with distilled water and filter sterilize. The solution should be used soon after preparation. Discard any unused solution.

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