Entamoeba histolytica HU-1:MUSC

Catalog No. NR-2592

(Derived from ATCC® 30922™)

For research use only. Not for human use.

Contributor:

ATCC®

Product Description:

Protozoa Classification: *Entamoebidae*, *Entamoeba*
Agent: *Entamoeba histolytica*
Strain: HU-1:MUSC
Original Source[1]: Isolated by W. B. Lushbaugh and F. E. Pittman from the feces of an infant female asymptomatic cyst passer in South Carolina in 1976
Comments: *Entamoeba histolytica* HU-1:MUSC[1] was deposited at ATCC® in 1981 by Dr. Louis S. Diamond[2-4], Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland. This culture contains mixed, unidentified bacteria that are required for growth of the *Entamoeba histolytica* culture.

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.[5]

Material Provided:

Each vial of NR-2592 contains approximately 0.5 mL of culture in cryopreservative. Please see Appendix I below for cryopreservation instructions.

Packaging/Storage:

NR-2592 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:

Note: For best results, please order two vials of NR-2592. One vial will be used to prepare bacterial flora (needed for *Entamoeba* growth) and the second vial for propagation of the protozoa.

Growth Media:

ATCC medium 1171: contains 3% heat-inactivated bovine serum (HIBS). Penicillin and streptomycin (ATCC® 30-2300™) can be added if bacterial density becomes too high.

Incubation:
Temperature: 35–37°C
Atmosphere: Xenic and microaerophilic

Propagation:

1. One day prior to initializing propagation of *Entamoeba histolytica* HU-1:MUSC, grow a preparation of bacteria flora using one of the frozen vials of NR-2592. Place the vial in a 35–37°C water bath for 2 to 3 minutes, until thawed. Note: Manipulations with the frozen vial should be done quickly to avoid warming the culture at a suboptimal rate.
   a. Inoculate each of 2 tubes of ATCC medium 1171 containing 3% HIBS without antibiotics (8 mL per tube) with a loopful of NR-2592.
   b. Incubate at a 15° horizontal slant for 24 hours at 35–37°C.
   c. Reduce the volume of one of the two cultures to 4.1 mL and add 0.9 mL of HIBS (total = 5 mL bacterial-enriched culture in ATCC medium 1171 containing 20% HIBS). Invert gently to mix.
   d. Centrifuge the bacterial-enriched cultures at 100-200 x g for 5 minutes to remove the starch from the media.
2. Aseptically add 0.5 mL of the bacteria-enriched culture containing 20% HIBS from step 1 to the second frozen vial of NR-2592. Place the vial in a 35–37°C water bath for 2 to 3 minutes, until thawed. Note: Manipulations with the frozen vial should be done quickly to avoid warming the culture at a suboptimal rate.
3. Transfer the vial contents to a glass one-dram (3.5 mL) screw-capped vial.
4. Add 2.5 mL of the bacteria-enriched culture containing 20% HIBS prepared in step 1. Tighten the cap and incubate at a 15° horizontal slant for 2–4 hours at 35–37°C.
5. Ice the vial for 10 minutes and gently invert 10 times. Centrifuge at 100-200 x g for 5 minutes.
6. Carefully aspirate the supernatant leaving the pelleted material containing the *Entamoeba* (and starch from the m edia).

Replace the supernatant in the dram vial containing the pellet with 3 mL of bacteria-enriched culture containing 3% HIBS prepared in step 1. Gently invert 10 times.

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Supplement with penicillin and streptomycin (ATCC® 30-2300™) if the bacterial density becomes too high.

7. Incubate the dram vial at a 15° horizontal slant at 35–37°C with the cap screwed on tightly. Observe the culture daily until trophozoites are observed (1-3 days).

If additional trophozoites are needed, transfer 2 mL from the dram vial to a new dram vial. Add 1.5 mL of bacteria-enriched culture containing 3% HIBS prepared in step 1. Gently invert 10 times. Supplement with penicillin and streptomycin (ATCC® 30-2300™) if the bacterial density becomes too high.

8. Incubate the second dram vial at a 15° horizontal slant at 35–37°C with the cap screwed on tightly. Observe the culture daily until adequate trophozoites are observed (1-3 days).

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* HU-1:MUSC, NR-2592."

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. Dykes, A. C., et al. "Extraintestinal Amebiasis in Infancy: Report of Three Patients and Epidemiologic Investigations of Their Families." Pediatrics 65 (1980): 799–803. PubMed: 7367088.
2. 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.
3. 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.
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5. Tanyuksel, M. and W. A. Petri. "Laboratory Diagnosis of Amebiasis." Clin. Microbiol. Rev. 16 (2003): 713–729. PubMed: 14557296.
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 10N 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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