Entamoeba histolytica, Strain 103:NIH (xenic)

Catalog No. NR-20085

For research use only. Not for human use.

Contributor:
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Manufacturer:
BEI Resources

Product Description:
Protozoa Classification: *Entamoebidae*, *Entamoeba*
Species: *Entamoeba histolytica*
Strain: 103:NIH
Original Source: *Entamoeba histolytica (E. histolytica)*, strain 103:NIH was isolated in September 1941 from a human case of amebic dysentery in India.[1,2]

E. histolytica is a pathogenic protozoan parasite and causative agent of amebiasis, an intestinal infection that predominantly infects humans and other primates in developing countries, with symptoms ranging from asymptomatic colonization to extraintestinal, dissiminated disease.[3,4,5] The *E. histolytica* life cycle consists of a highly resistant environmental cyst with a protective, chitin-rich cell wall and a dividing trophozoite, which establishes infection through excystation in the colon.[4,5] Infection occurs through shedding of cysts in feces and the ingestion of cysts via contaminated water and vegetables.[6] *E. histolytica* has been shown to cause host tissue damage through amoebic trogocytosis in a mouse model.[7]

Material Provided:
Each vial of NR-20085 contains approximately 0.5 mL of culture in cryopreservative. Please refer to the Certificate of Analysis for the specific culture media used for each lot and refer to Appendix I for cryopreservation instructions.

Note: NR-20085 is a xenic culture.

Packaging/Storage:
NR-20085 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 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:
TYGM-9 supplemented with 20% heat-inactivated adult bovine serum (HIBS) and 75 I.U./mL penicillin and 75 µg/mL streptomycin to control bacterial density.
Incubation:
Temperature: 35°C
Atmosphere: Xenic and microaerophilic
Propagation:
1. Aliquot TYGM-9 medium in a 15 mL test tube and allow the starch to settle to the bottom of the tube. Transfer the medium without the starch sediment to a new tube and supplement with 20% heat-inactivated bovine serum (HIBS).
2. To establish a culture from the frozen state, aseptically add 0.5 mL of growth medium containing 20% HIBS without antibiotic to the frozen vial of NR-20085. Place the vial in a 35°C to 37°C water bath for 2 to 3 minutes, until thawed.
   Note: Manipulations with the frozen vial should be done quickly to avoid warming of the culture at a suboptimal rate.
3. Transfer the vial contents to a glass one-dram (3.5 mL) screw-capped vial and add 2.5 mL of additional TYGM-9 containing 20% HIBS in a dropwise fashion. Tighten the cap and incubate in an upright position for 2 to 3 hours at 35°C.
4. Ice the vial for 10 minutes and gently invert 10 times. Centrifuge at 100 to 200 × g for 5 minutes.
5. Aspirate the supernatant leaving approximately 0.5 mL. Do not aspirate the pelleted material.
6. Replace the supernatant with 3.0 mL of TYGM-9 medium that has not been supplemented with 20% HIBS. Add 75 I.U./mL of penicillin and 75 µg/mL of streptomycin to control bacterial growth.
7. Incubate the dram vial at a 15° horizontal slant at 35°C with the cap screwed on tightly. Observe the culture daily and transfer when culture has reached early stationary phase as determined by large numbers of trophozoites.
8. To transfer the culture, ice the one-dram vial for 10 minutes, invert 10 times, and aseptically transfer 0.1 mL and 0.3 mL aliquots to two 16 × 125 mm test tubes with TYGM-9 medium containing antibiotics to control bacterial growth. Incubate the test tubes at a 15° horizontal slant at 35°C with the cap screwed on tightly. Observe the culture daily and transfer as needed.

Please refer to Appendix I for cryopreservation instructions.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Entamoeba histolytica*, Strain 103:NIH (xenic), NR-20085."

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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, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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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 × g for 5 minutes.
5. While cells are centrifuging, prepare the CPMB-5 Cryoprotective Solution:
    a. Add 1 mL of DMSO to a 16 × 125 mm screw-capped test tube and place on ice until solidified.
    b. Add 0.8 mL of 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 mL of the CPMB-2 Basal Solution and mix.
    e. Add 2 mL heat inactivated bovine serum (HIBS) 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 5×10^5 and 1×10^6 cells/mL using fresh media. If the cell concentration is lower than 5×10^5 cells/mL, centrifuge the cell suspension, remove the supernatant, and resuspend the pellet in a volume that will yield a concentration between 5×10^5 and 1×10^6 cells/mL.
8. After the cell concentration is adjusted, centrifuge at 200 × 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 sterile plastic 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 L

Add the ingredients in the order listed above to the distilled water and mix. Adjust the pH to 6.8 and autoclave for 20 minutes at 121°C.

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

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 using a 0.2 µm filter. The solution should be used soon after preparation. Discard any unused solution.

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