# *Entamoeba histolytica,* Strain IP:1182:2 (xenic)

## Catalog No. NR-20088

## 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: IP:1182:2 (xenic)
Original Source: *Entamoeba histolytica* (*E. histolytica*), strain IP:1182:2 was isolated in 1982 from a sigmoidoscopy of an adult male Caucasian Canadian with amoebic dysentery following a brief visit to Honduras.[1-3]

*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.[4]

### Material Provided:
Each vial of NR-20088 contains approximately 0.5 mL of culture in cryopreservative. Please see Appendix I below for cryopreservation instructions.

Note: NR-20088 is a xenic culture.

### Packaging/Storage:
NR-20088 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:
TYSGM-9 supplemented with 5% heat-inactivated adult bovine serum (HIBS) and 1,000 I.U./mL penicillin and 1,000 µg/mL streptomycin to control bacterial density. Please see Appendix II for TYSGM-9 preparation instructions.
Incubation:
Temperature: 35°C to 37°C
Atmosphere: Xenic and microaerophilic
Propagation:
1. To establish a culture from the frozen state, aseptically add 0.5 mL of growth medium containing 20% HIBS but free of antibiotic to the frozen vial of NR-20088. 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.
2. Transfer the vial contents to a glass one-dram (3.5 mL) screw-capped vial and add 2.5 mL of additional TYSGM-9 containing 20% HIBS. Tighten the cap and incubate in an upright position for 2 to 4 hours at 35°C to 37°C.
3. Ice the vial for 10 minutes and gently invert 10 times. Centrifuge at 200-350 x g for 5 minutes.
4. Carefully aspirate the supernatant without disturbing the cell pellet and transfer to a second dram vial. Resuspend the pelleted material containing the *Entamoeba* and starch with 3 mL of growth medium containing 5% HIBS. Gently invert 10 times.
5. Incubate the two dram vials at a 15° horizontal slant at 35°C to 37°C with the caps screwed on tightly. Observe the culture harboring *Entamoeba* daily until trophozoites are observed (1 to 3 days). If trophozoites are not apparent after 3 days, re-feed the culture with the bacteria-enriched culture prepared in step 4 supplemented with 5% HIBS. Treat the culture with penicillin/streptomycin solution if the bacterial density becomes too high.
6. Subculture by transferring 2 mL of culture to a new dram vial. Add 1.5 mL of bacteria-enriched culture prepared in step 4 supplemented with 5% HIBS. Gently invert 10 times. Supplement with penicillin/streptomycin solution if the bacterial density becomes too high.
7. Incubate the second dram vial at a 15° horizontal slant at 35°C to 37°C with the cap screwed on tightly. Observe the culture daily until trophozoites are observed (1 to 3 days).

Please see Appendix I below for cryopreservation instructions.

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## Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Entamoeba histolytica,* Strain IP:1182:2 (xenic), NR-20088."

## 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 http://www.cdc.gov/biosafety/publications/bmbl5/index.htm.

## Disclaimers:

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## References:

1. Chadee, K. and E. Meerovitch. "*Entamoeba histolytica*: Lymphoreticular Changes in Gerbils (*Meriones unguiculatus*) with Experimentally Induced Cecal Amebiasis." J. Parasitol. 71 (1985): 566-575. PubMed: 2865345.
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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 CPMB-5 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 |

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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## APPENDIX II: TYSGM-9

Prepare ingredients in order listed below:

| | |
|---|---|
| $K_2HPO_4$ | 2.8 g |
| $KH_2PO_4$ | 0.4 g |
| NaCl | 7.5 g |
| Casein digest peptone | 2.0 g |
| Yeast Extract | 1.0 g |
| Distilled water | to 950.0 mL |

Bring solution to 950 mL with distilled water. Dispense the solution in 95 mL aliquots and add 0.2 g bovine gastric mucin to each bottle. Autoclave for 15 minutes at 121°C with 15 lbs. pressure and store refrigerated at 4°C. Before use, add 0.1 mL of a filter sterilized 5% stock of Tween 80 in distilled water and 5 mL of heat inactivated adult bovine serum. Dispense 8 mL aliquots into 16 x 125 mm culture tubes.

Additional Xenic Culture information is available at the: *Entamoeba* Home Page.

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