Entamoeba invadens, Strain IP-1 (axenic)

Catalog No. NR-15226
(Derived from ATCC® 30994™)

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Contributor:
ATCC®

Manufacturer:
BEI Resources

Product Description:
Protozoa Classification: *Entamoebidae*, *Entamoeba*
Species: *Entamoeba invadens*
Strain: IP-1
Source: *Entamoeba invadens (E. invadens)*, strain IP-1 was originally isolated by Dr. E. Meerovitch from a green water snake *(Natrix cyclopion)* in Florida in 1952.[1]
Comments: *E. invadens,* strain IP-1 was originally deposited to ATCC® in 1982 by Dr. Louis S. Diamond, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA. The whole genome shotgun sequence of *E. invadens,* strain IP-1 is available (GenBank: AANW00000000).

Protozoan parasites of the genus *Entamoeba* infect a large number of vertebrates. *E. histolytica* is a human parasite that is the causative agent of intestinal amebiasis. The disease-causing ameboid form of the parasite differentiates into a thick-walled cyst for transmission from person to person.[2] *E. invadens* is a parasite of reptiles and is the only species that can be induced to form cysts in axenic culture. *E. invadens* is a useful model for the study of the mechanisms of encystment and excystment for the human parasite.[3,4]

Material Provided:
Each vial of NR-15226 contains approximately 0.5 mL of cells in cryopreservative [10% dimethylsulfoxide (DMSO)]. Please refer to Appendix I for cryopreservation instructions.

Note: NR-15226 is an axenic culture.

Packaging/Storage:
NR-15226 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:
Liver Digest – Yeast Extract – Iron (LYI) *Entamoeba* medium supplemented with 10% heat-inactivated adult bovine serum (HIBS) and Diamond's Vitamin solution or equivalent (Appendix II)
Incubation:
Temperature: 25°C
Atmosphere: Microaerophilic
Propagation:
Note: *E. invadens* may be slow to recover from the frozen state. It is recommended to leave the culture undisturbed the first 3-to-4 days after thawing.

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 × 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 25°C. Observe the culture daily and subculture when peak density is observed.
4. If no viable cells are observed after 3-to-4 days, prepare a subculture by setting the test tube in an upright position for 10 minutes. Remove 1 mL-to-2 mL of cellular material from the bottom of the tube and transfer it to a fresh tube with fresh culture media. Add fresh medium to the original test tube. Transfer both tubes to a 25°C incubator, and monitor daily for growth.

Maintenance:

1. When the culture is at or near peak density, ice the culture for 10 minutes and gently invert 20 times.
2. Add 12 mL of freshly prepared growth media to two sterile tubes.
3. Aseptically transfer a 100 µL to 250 µL aliquot of *E. invadens*, strain IP-1 culture to the tubes prepared in step 2.
4. Screw the cap on tightly and incubate at a 15° horizontal slant at 25°C. Observe the culture daily and subculture when peak density is observed.

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 invadens,* Strain IP-1, NR-15226."

Biosafety Level: 1
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.

Disclaimers:

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References:

1. Meerovitch, E. “Some Biological Requirements and Host-Parasite Relations of *Entamoeba invadens*.” Can. J. Zool. 36 (1958): 513-523.
2. Eichinger, D. “Encystation of *Entamoeba* Parasites.” BioEssays 19 (1997): 633–639. PubMed: 9230696.
3. Vazquezdelara-Cisneros, L. G. and A. Arroyo-Begovich. “Induction of Encystation of *Entamoeba invadens* by Removal of Glucose from the Culture Medium.” J. Parasitol. 70 (1984): 629-633. PubMed: 6512629.
4. Sanchez, L., V. Enea, and D. Eichinger. “Identification of a Developmentally Regulated Transcript Expressed during Encystation of *Entamoeba invadens*.” Mol. Biochem. Parasitol. 67 (1994): 125-135. PubMed: 7838173.
5. Petri, W. A., Jr. and U. Singh. “Diagnosis and Management of Amebiasis.” Clin. Infect. Dis. 29 (1999): 1117-1125. PubMed: 10524950.
6. Gillin, F. D. and L. S. Diamond. “Clonal Growth of *Entamoeba histolytica* and Other Species of *Entamoeba* in Agar.” J. Protozool. 25 (1978): 539-543. PubMed: 216801.

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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.

APPENDIX II: LIVER DIGEST – YEAST EXTRACT – IRON (LYI) *ENTAMOEBA* MEDIUM (ATCC® MEDIUM 2154)

1. Prepare the 10× Glucose Buffer Stock Solution (see recipe below) and filter sterilize using a 0.2 µm filter.

2. Prepare the LYI Base Stock Solution (see recipe below), by dissolving the dry ingredients of the LYI Base Stock in 600 mL of distilled water in the order indicated. Adjust the final volume to 780 mL with distilled water. Adjust pH to 6.8 with 1 N NaOH. Autoclave for 20 minutes at 121°C, and allow to cool.

10× Glucose Buffer Stock Solution	
K_2HPO_4	1.0 g
KH_2PO_4	0.6 g
Glucose	10.0 g
Distilled water	100 mL

LYI Base Stock	
NaCl	1.0 g
Yeast Extract	25.0 g
Neutralized Liver Digest	5.0 g
L-Cysteine • HCl	1.0 g
Ascorbic Acid	0.2 g
Ferric Ammonium Citrate	22.8 mg
Distilled water to	780 mL

3. Prepare the LYI Broth (see recipe below), by aseptically adding 100 mL of the 10× Glucose Buffer Stock Solution to 780 mL of cooled LYI Base Stock Solution. Osmolarity should be 380 milliosmols/kg; adjust by increasing or decreasing NaCl. LYI Broth can be stored at least 6 months at -20°C.

LYI Broth	
LYI Base Stock Solution	780 mL
10× Glucose Buffer Stock Solution	100 mL

4. Prepare each of the three water-soluble vitamin stock solutions listed below:

Water Solution A	
Niacin	62.5 mg
p-Aminobenzoic acid	12.5 mg
Distilled water to	150 mL

Dissolve solid ingredients in boiling distilled water and restore the final volume to 150 mL.

Water Solution B	
Niacinamide	62.5 mg
Pyridoxine hydrochloride	62.5 mg
Thiamine hydrochloride	25.0 mg
Calcium pantothenate	25.0 mg
i-Inositol	125.0 mg
Choline chloride	1250.0 mg
Distilled water to	150 mL

Dissolve solid ingredients in 125 mL distilled water, then bring the final volume to 150 mL.

Water Solution C	
Riboflavin	62.5 mg
Distilled water to	150 mL

Add riboflavin to 75 mL of distilled water and add 0.1 N NaOH dropwise until the riboflavin is fully dissolved. Bring the final volume to 100 mL with distilled water.

5. Prepare the Water-Soluble Vitamins Solution by combining each of the three water-soluble vitamin stock solutions prepared above. Bring the final volume to 500 mL with distilled water.

Water Soluble Vitamins	
Water Solution A	150 mL
Water Solution B	150 mL
Water Solution C	100 mL
Distilled water to	100 mL

If the mixture appears turbid, it should not be discarded. Development of turbidity is an indication that an excess of NaOH has been used in the preparation of one of the stock solutions (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).

6. Prepare the Biotin Solution following the recipe below:

   Biotin Solution
   Biotin 30 mg
   Distilled water to 300 mL
   Add biotin to 200 mL of distilled water and add 0.1 N NaOH dropwise until the biotin is fully dissolved. Bring the final volume to 300 mL with distilled water.

7. Prepare the Folic Acid Solution following the recipe below:

   Folic Acid Solution
   Folic Acid 30 mg
   Distilled water to 300 mL
   Add folic acid to 200 mL of distilled water and add 0.1 N NaOH dropwise until the folic acid is fully dissolved. Bring the final volume to 300 mL with distilled water.

8. Prepare the each of the three Lipid Stock Solutions following the recipes listed below:

   Lipid Solution A
   Vitamin D_2 (calciferol) 300 mg
   Ethyl alcohol 9.5% (v/v) 63 mL
   Vitamin A (crystalline alcohol) 300 mg
   Dissolve vitamin D_2 in ethyl alcohol, then add vitamin A.

   Lipid Solution B
   Vitamin K (menadione sodium bisulfite) 60 mg
   Tween 80 aqueous solution 5% (v/v) 300 mL

9. Prepare the Lipid-Soluble Vitamins A, D and K Solution following the recipe below by combining Lipid Solutions A and B prepared above:

   Lipid-Soluble Vitamins A, D and K
   Lipid Solution A 60 mg
   Lipid Solution B 300 mL

10. Prepare the Vitamin E Stock Solution following the recipe below:

    Vitamin E Stock Solution
    Vitamin E (alpha tocopherol acetate) 25 mg
    Distilled water 250 mL

11. Prepare the Diamond's Vitamin Solution 107 following the recipe below, and sterile filter using a 0.22 µm filter. The complete, clear solution may be stored at -22°C. Thaw and allow to adjust to room temperature before use.

    Diamond's Vitamin Solution 107
    Water-Soluble B Vitamins 500 mL
    Biotin Solution 250 mL
    Folic Acid Solution 250 mL
    Lipid-Soluble Vitamins A, D & K 2500 mL
    Vitamin E Solution 100.0 mL

12. Aseptically prepare the complete LYI *Entamoeba* medium following the recipe below:

    LYI *Entamoeba* Medium
    LYI Broth 88 mL
    Diamond's Vitamin Solution 107 2 mL
    Heat-Inactivated Bovine Serum (HIBS) 10 mL

    Mix thoroughly and distribute into 13 mL aliquots to 16 × 125 mm screw-capped borosilicate glass test tubes. Store at 5°C to 9°C in the dark with the caps screwed on tightly. Use within 7 to 10 days.