ATCC

Product Sheet

Entamoeba sp. (ATCC® 50311™)

Please read this FIRST

Biosafety Level
1

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Entamoeba sp.* (ATCC® 50311™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Strain Designation: NIH:1091:1
Deposited Name: *Entamoeba* sp.
Depositor: CG Clark, LS Diamond
Isolation:
green iguana, Iguana iguana, at National Zoological Park, Washington, DC, 1991

Propagation

Growth Conditions
Temperature: 25.0°C
Duration: anaerobic; grown with bacteria
Protocol: ATCCNO: 30131 SPEC: This culture is xenic; i.e., it contains mixed unidentified bacteria, some or all of which serve as food for the amoeba. 1) A growing culture is shipped in a 16 X 125 mm screw-capped test tube filled to within approximately two centimeters of the top with medium, a configuration which enhances survival in transit. 2) Immediately upon receipt of the culture, place it on a 15-degree slant at 35C. Allow the culture to remain undisturbed for at least three hours. 3) Observe the culture with an inverted microscope. Attached trophozoites should be evident. Reduce the volume of the culture to approximately 9 ml. 4) Centrifuge the removed culture fluid at 500 x g for five minutes. Under these conditions any trophozoites in suspension will be pelleted to the bottom of the tube. 5) Inoculate two fresh tubes of ATCC medium 1171 (available from ATCC as item IV-1171) with 0.25 ml of the supernatant derived in step 4 and incubate the tubes at 35C. These tubes will serve as preinoculated bacterized culture tubes. Preinoculation of medium with bacteria prior to subcultivation of Dientamoeba and bacterized Entamoeba strains allows for better growth. 6) Divide the remainder of the supernatant from step 4 into two equal aliquots in 16 X 125 mm screw-capped test tubes. Increase the volume of each tube to approximately 9 ml with fresh ATCC medium 1171. 7) Ice the parent shipped culture for five minutes, invert the tube 20 times and transfer 0.5- and 1.0-ml aliquots to the tubes just set up in step 6. 8) Incubate all cultures at 35C. Transfer cultures when they reach early stationary phase. The transfer interval will depend on the quality of the culture medium used. Inoculate bacterized culture tubes at least one day prior to subcultivation of Dientamoeba and bacterized Entamoeba strains. 9) In general, addition of penicillin G at 75 U/ml and streptomycin at 75 mcg/ml to ATCC medium 1171 may be necessary if the bacterial density is too high.

Medium
ATCC® Medium 1171: TYGM-9 medium

Instructions for Complete Medium
ATCC Medium 1171

Culture Maintenance
1. Ice a test tube culture at or near peak density for 10 minutes, invert 20 times and aseptically transfer a 0.1 and 0.3 ml aliquot to a fresh tube of ATCC Medium 1171. Note: Growth is often better if the growth medium is inoculated with bacteria at least 24 hrs before inoculating with amoebae.
2. Screw cap on tightly and incubate on a 15° horizontal slant at 25°C.

Cryopreservation

CPMB-5 Cryoprotective Solution

DMSO	1.0 ml
2.5 M Sucrose	0.8 ml
L-Cysteine/Ascorbic Acid Solution	0.2 ml
CPMB-2 Basal Solution	6.0 ml
HIBS	2.0 ml

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

Autoclave for 15 minutes.
L-Cysteine/Ascorbic Acid Solution

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L-Cysteine-HCL	1.0 g
Ascorbic Acid	0.1 g
Distilled water	10.0 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 10N NaOH (approximately 0.7 ml). Adjust final volume to 10 ml with distilled water and filter sterilize. Solution should be used soon after preparation. Discard any unused solution.

1. Harvest cells from several cultures that are in the late logarithmic to early stationary phase of growth. Place culture vessels on ice for 10 min.
2. Invert tubes 20 times and centrifuge at 200 x g for 5 min.
3. 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 solution, remove from ice and invert until the DMSO is liquefied. Return to ice bath.
c) Add 0.2 ml of the L-Cysteine/Ascorbic Acid Solution to the DMSO solution and mix.
d) Add 6.0 ml of the CPMB -2 Basal Solution and mix.
e) Add 2.0 ml HIBS and mix.

4. Resuspend the cell pellets and pool to a final volume of approximately 10 ml with the supernatant. Make a determination of the cell density and adjust the concentration of the cells between 5 x 10^5/ml - 1 x 10^6/ml using fresh medium. If the cell concentration is below 5 x 10^6/ml, centrifuge the cell suspension and resuspend the pellet in a volume that will yield the desired concentration.
5. After the cell concentration is adjusted, centrifuge as in step 2.
6. Remove as much supernatant as possible and determine the volume removed.
7. Resuspend the cell pellet with a volume of the cryoprotective solution equal to the volume of the supernatant removed. Invert the tube several times to obtain a uniform cell density.
8. Dispense 0.5 ml aliquots into 1.0 - 2.0 ml plastic sterile cryules (special plastic vials for cryopreservation).
9. Place the vials in a controlled rate freezing unit. Use the following cooling cycle: From room temperature cool at
-10°C/min to the heat of fusion; from the heat of fusion to
-40°C, cool at -1°C/min. At -40°C plunge into liquid nitrogen. The cooling cycle should be initiated no less than 15 and no more than 30 minutes after the addition of DMSO to the cell preparation.
10. Store ampules in a liquid nitrogen refrigerator until needed.
11. One day before thawing a frozen ampule inoculate two tubes of ATCC medium 1171 with the bacterial flora only. Incubate the tube on a 15° horizontal slant at 35°C.
12. On the following day reduce the volume of the culture from 8.5 to 8.25 ml and add 1.75 ml of HIBS. Invert gently several times to mix.
13. Remove the frozen ampule from liquid nitrogen and flame gently at the base of the cap. Remove the cap and aseptically add 0.5 ml of the modified medium prepared in step 12. Place in a 35°C water bath until thawed (2-3 min). Note: Manipulations of the ampule before placing in the water bath should be done as quickly as possible to avoid warming of the contents at a suboptimal rate.
14. Transfer contents of the thawed ampule to a dram screw-capped vial (vial holds approximately 4.0 ml).
15. Add 2.5 ml of serum supplemented medium prepared in step 14. Tighten the cap and incubate on a 15° horizontal slant at 25°C for 2-3 hours.
16. Ice the vial for 10 minutes, invert gently 10 times, and centrifuge at 100-200 x g for 5 min.
17. Aspirate the supernatant leaving approximately 0.5 ml. Note: Do not aspire the pelleted material.
18. Replace the supernatant with 3.0 ml of ATCC medium that has been inoculated with the bacterial flora.
19. Incubate the vial on a 15° horizontal slant at 25°C with the cap screwed on tightly. Observe the culture daily and transfer when many trophozoites are observed.

References

References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media

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may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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Additional information on this culture is available on the ATCC web site at www.atcc.org.

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