ATCC

Product Sheet

Entamoeba moshkovskii (ATCC® 50415™)

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 moshkovskii* (ATCC® 50415™)

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: AP-1
Deposited Name: *Entamoeba moshkovskii* Tshalaia
Depositor: SA Schaffer
Isolation:
pool in rock crevice two feet above the splash zone; remains of a decaying bird were present in the pool, Appledore Island, ME, 1993

Propagation

Growth Conditions
Temperature: 25.0°C
Duration: anaerobic
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.

Instructions for Complete Medium
ATCC Medium 1873

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 1873.
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 Base Solution	6.0 ml
Heat-inactivated bovine serum	2.0 ml
CPMB #2 Basal Solution	
Casein Digest Peptone (BBL)	40.0 g
Yeast Extract	20.0 g
K_2HPO_4	1.0 g
KH_2PO_4	0.6 g
NaCl	2.0 g
Distilled water	1.0 L
(Autoclave the solution)	
L-Cysteine/Ascorbic Acid Solution	
L-Cysteine-HCL	1.0 g
Acorbic Acid	0.1 g

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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.

1. Harvest cells from several cultures which 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 the DMSO in a 16 x 125 mm screw-capped 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 heat-inactivated bovine serum 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 106/ml, centrifuge the cell suspension and resuspend the pellet in a volume the 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 vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through heat of fusion. At -40°C plunge ampules into liquid nitrogen.
10. Store ampules in a liquid nitrogen refrigerator until needed.
11. To establish a culture from the frozen state, place an ampule in a 35°C water bath, until thawed (2-3 min). Immerse the vial just sufficient to cover the frozen material. Do not agitate the ampule.
12. Transfer contents of thawed ampule to a 16 x 125 mm screw-capped test tube containing 13 ml of ATCC medium 1978.
13. Screw cap on tightly and incubate at a 15° horizontal slant at 25°C. 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 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

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this

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product. The MTA is also available on our Web site at www.atcc.org

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

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