



Product Sheet

Entamoeba moshkovskii (ATCC® 50566™)

Please read this **FIRST**

Storage Temp.
Frozen Cultures:
-70°C for 1 week;
liquid N₂ vapor
for long term
storage



Freeze-dried Cultures:
2-8°C

Live Cultures:
See Protocols
section for
handling
information



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® 50566™)

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

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Description

Strain Designation: 431
Deposited Name: *Entamoeba moshkovskii* Tshalaia
Depositor: LS Diamond
Isolation: Sewage sludge, Ontario, Canada

Propagation

Growth Conditions
Temperature: 25°C
Atmosphere: Anaerobic
Culture System: Xenic; grown with mixed bacteria

Medium
ATCC® Medium 1171: TYGM-9 medium

Protocols

Handling of Live Cultures

This strain is routinely shipped as a growing culture with bacteria in a glass 16 x 125 mm screw-capped test tube. The volume of the cell suspension is approximately 15 mL. When the culture arrives remove it promptly from the shipping container. **Do not store the culture at refrigeration temperatures before handling.** To assure viability, immediately incubate culture at a 15° horizontal slant at 35°C for at least three hours before observing the culture. There should be numerous active trophozoites attached to the tube. If the numbers are low the culture may have been exposed to temperature extremes in transit. Regardless of the state of the culture, remove 6 mL of fluid and centrifuge at 500 x g for 5 minutes. Inoculate 2 fresh tubes of media with 0.25 mL of supernatant (preinoculated bacterized culture tubes allow for better growth). Divide the remainder of the supernatant in two equal aliquots in 16 x 125 mm screw-capped tubes and bring the volumes up to 8 mL with fresh ATCC Medium 1171. Ice the parent culture 5 minutes, invert 20 times and transfer 0.5 and 1.0 mL aliquots to the test tubes containing the equal volumes of supernatant. Incubate the cultures at a 15°C horizontal slant at 35°C. Transfer when the cultures reach the early stationary phase. In general, addition of penicillin G at 75 U/mL and streptomycin at 75 µg/mL to ATCC Medium 1171 may be necessary if the bacterial density is too high. **NOTE:** Inoculate bacterized culture tubes at least one day prior to subcultivation and prior to addition of antibiotics, if used.

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 35°C.

Cryopreservation

Reagents

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
K₂HPO₄ 1.0 g
KH₂PO₄ 0.6 g
NaCl 2.0 g
Distilled water 1.0 L
Autoclave for 15 minutes.

L-Cysteine/Ascorbic Acid Solution
L-Cysteine-HCL 1.0 g



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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.

Harvest and Preservation

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 10⁶/mL - 1 x 10⁹/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 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 35°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 35°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

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

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