



Entamoeba moshkovskii **Tshalaia**

50415™

Product Sheet

Description

Strain designation: AP-1

Deposited As: *Entamoeba moshkovskii* Tshalaia

Type strain: No

Storage Conditions

Product format: Test tube

Intended Use

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

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always

used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Medium:

ATCC Medium 1873: Seawater microaerophile medium

Instructions for complete medium: ATCC Medium 1873

Temperature: 25°C

Atmosphere: Anaerobic

Culture system: Xenic

Handling Procedures

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.

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Reagents for 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 ₂ HPO ₄	1.0 g
KH ₂ PO 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
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.

Cryopreservation:

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.

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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 $5 \times 10^5/\text{ml}$ - $1 \times 10^6/\text{ml}$ using fresh medium. If the cell concentration is below $5 \times 10^6/\text{ml}$, 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 cryovials (special plastic vials for cryopreservation).
9. Place vials in a controlled rate freezing unit. From room temperature cool at $-1^\circ\text{C}/\text{min}$ to -40°C . If freezing unit can compensate for the heat of fusion, maintain rate at $-1^\circ\text{C}/\text{min}$ 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.

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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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Entamoeba moshkovskii* Tshalaia (ATCC 50415)

References

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

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