Entamoeba dispar Brumpt

PRA-260™

Description

Strain designation: SAW 760
Deposited As: *Entamoeba dispar* Brumpt
Type strain: No

Storage Conditions

Product format: Frozen
Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

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 2692: Modified LYI Entamoeba Medium
Instructions for complete medium: ATCC Medium 2692
This culture is monoxenic, cultivated with *Crithidia fasciculata* (ATCC 50083) as a food source
Temperature: 35°C
Atmosphere: Microaerophilic
Culture system: Xenic
Incubation: With *Crithidia fasciculata* (ATCC 50083)

Handling Procedures

Establishing a culture of *Entamoeba dispar* from a frozen ampule:

Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampules may be stored at or below -70°C for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally -20°C).** Storage of frozen material at this temperature will result in the death of the culture.

1. To thaw a frozen ampule, place in a 35°C water bath, until thawed (2-3 min). Immerse the ampule just sufficiently to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer contents to a glass screw-capped tube containing 12 mL ATCC Medium 2692 and 1 mL from a growing culture of *Crithidia fasciculata* (see below). Screw cap on tightly and incubate on a 15° horizontal slant at 35°C.

Establishing a culture of *Crithidia fasciculata* from a frozen ampule:
Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampoules may be stored at or below -70°C for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally -20°C).** Storage of frozen material at this temperature will result in the death of the culture.

1. To thaw a frozen ampule, place it in a 35°C water bath such that the lip of the ampule remains above the water line. Thawing time is approximately 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after thawed.
2. Immediately after thawing, aseptically transfer contents to a 16 x 125 mm screw-capped test tube containing 5 mL ATCC Medium 355.
3. Incubate upright at 25°C with cap screwed on tightly.

Culture maintenance: Maintenance of *Entamoeba dispar:*

1. Ice culture at or near peak density for 10 min.
2. Gently invert culture 20 times.
3. Remove 1 mL of medium from each of two freshly prepared (no older than 7-10d) tubes of ATCC medium 2692 and add 1 mL of *Crithidia fasciculata* to each.
4. Aseptically transfer a 0.1 and 0.25 mL aliquot of *Entamoeba dispar* to the tubes prepared in step 3.
5. Screw caps on tightly and incubate at a 15° horizontal slant at 35°C.

6. Subculture when many trophozoites are observed (typically every 2-4 days). The transfer interval will depend on the quantity of the inoculum and the quality of the medium. This should be empirically determined by examining the culture on a daily basis until the growth cycle has stabilized. Do not allow the culture to overgrow. The culture crashes soon after reaching peak density.

Maintenance of *Crithidia fasciculata:*

1. When the culture is at or near peak density, vigorously agitate the culture.
2. Transfer approximately 0.1 mL to a fresh tube containing 5 mL of fresh ATCC medium 355.
3. Incubate upright at 25°C with cap screwed on tightly.
4. Transfer every 14 days.

See product sheet for ATCC® 50083™.

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 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
L-Cysteine-HCL, 1.0 g
Acorbic 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.

Cryopreservation:

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 10^5/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. 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 sufficiently to cover the frozen material. Do not agitate the ampule.
12. Transfer contents of thawed ampule to a 16 x 125 mm screw-capped borosilicate glass test tube containing 12 mL of ATCC medium 2692 and 1 mL

from a growing culture of *Crithidia fasciculata*.

13. Screw cap on tightly and incubate at a 15° horizontal slant at 35°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 dispar* Brumpt (ATCC PRA-260)

References

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

Warranty

The product is provided 'AS IS' and the viability of ATCC® products is warranted for 30 days from the date of shipment, provided that the customer has stored and handled the product according to the information included on the product information sheet, website, and Certificate of Analysis. For living cultures, ATCC lists the media formulation and reagents that have been found to be effective for the product. While other unspecified media and reagents may also produce satisfactory results, a change in the ATCC and/or depositor-recommended protocols may affect the recovery, growth, and/or function of the product. If an alternative medium formulation or reagent is used, the ATCC warranty for viability is no longer valid. Except as expressly set forth herein, no other warranties of any kind are provided, express or implied, including, but not limited to, any implied warranties of merchantability, fitness for a particular purpose, manufacture according to cGMP standards, typicality, safety, accuracy, and/or noninfringement.

Disclaimers

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. Any proposed commercial use is prohibited without a license from ATCC.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate or complete and the customer bears the sole responsibility of confirming the accuracy and completeness of any such information.

This product is sent on the condition that the customer is responsible for and assumes all risk and responsibility in connection with the receipt, handling, storage, disposal, and use of the ATCC product including without limitation taking all appropriate safety and handling precautions to minimize health or environmental risk. As a condition of receiving the material, the customer agrees that any activity undertaken with the ATCC product and any progeny or modifications will be conducted in compliance with all applicable laws, regulations, and guidelines. This product is provided 'AS IS' with no representations or warranties whatsoever except as expressly set forth herein and in no event shall ATCC, its parents, subsidiaries, directors, officers, agents, employees, assigns, successors, and affiliates be liable for indirect, special, incidental, or consequential damages of any kind in connection with or arising out of the customer's use of the product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.

Please see the material transfer agreement (MTA) for further details regarding the use of this product. The MTA is available at www.atcc.org.

Copyright and Trademark Information

© ATCC 2023. All rights reserved.
ATCC is a registered trademark of the American Type Culture Collection.

Revision

This information on this document was last updated on 2026-05-29

Contact Information

ATCC
10801 University Boulevard
Manassas, VA 20110-2209
USA
US telephone: 800-638-6597
Worldwide telephone: +1-703-365-2700
Email: tech@atcc.org or contact your local distributor