



Entamoeba dispar Brumpt

PRA-260™

Description

Strain designation: SAW 760

Deposited As: *Entamoeba dispar* Brumpt

Type strain: No

Storage Conditions

Product format: Test tube

Storage conditions: See handling procedure

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

Handling test tube cultures of monoxenic *Entamoeba dispar* strains upon arrival:

This strain is routinely shipped as a growing culture in a glass 16 x 125 mm screw-capped test tube. The volume of the cell suspension is approximately 15.5 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 on a 15° horizontal slant at 35°C for at least three hours before observing the culture. There should be numerous attached trophozoites. If the numbers are low the culture may have been exposed to temperature extremes in transit. Regardless of the state of the culture, ice the culture for 10 min. and gently invert 20 times. Aseptically transfer 0.5 and 0.1 mL aliquots to two 16 x 125 mm screw-capped test tubes each containing 12 mL of sterilized ATCC medium 2692 and 1 mL from a growing culture of *Crithidia fasciculata*. Incubate the parent and daughter cultures at a 15° horizontal slant with the caps on tightly at 35°C. See below for routine maintenance procedure.

Establishing cultures of *Crithidia fasciculata* from a frozen state:

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

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

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.

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 $5 \times 10^5/\text{mL}$ - $1 \times 10^6/\text{mL}$ using fresh medium. If the cell concentration is below $5 \times 10^5/\text{mL}$, 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 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 $-10^\circ\text{C}/\text{min}$ to the heat of fusion; from the heat of fusion to -40°C , cool at $-1^\circ\text{C}/\text{min}$. 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.

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