



Product Sheet

# *Entamoeba dispar* (ATCC®) 50631™)

Please read this **FIRST**



## 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 dispar* (ATCC® 50631™)

American Type Culture Collection  
PO Box 1549  
Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

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## Description

**Strain Designation:** CDC:0784:4

**Deposited Name:** *Entamoeba dispar* Brumpt

**Depositor:** LS Diamond

**Isolation:**

The strain grows only in the presence of *Crithidia fasciculata* ATCC 50083.

## Notes

This culture is monoxenic, cultivated with *Crithidia fasciculata* ATCC® 50083™ as a food source.

## Propagation

### Growth Conditions

**Temperature:** 35°C

**Atmosphere:** Anaerobic. Consult product sheet for culture protocol.

### Medium

ATCC® Medium 2154: LYI *Entamoeba* medium

### Instructions for Complete Medium

ATCC Medium PRA-2154 (Quality controlled freeze-dried lots of this medium are commercially available from ATCC).

## Protocols

### Establishing a culture of *Entamoeba dispar* from a frozen ampoule:

Frozen ampoules 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 ampoules 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 ampoule, place in a 35°C water bath, until thawed (2-3 min). Immerse the ampoule just sufficiently to cover the frozen material. Do not agitate the ampoule.
2. Immediately after thawing, aseptically transfer contents to a glass screw-capped tube containing 12 ml ATCC Medium 2154 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 ampoule:

Frozen ampoules 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 ampoules 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 ampoule, place it in a 35°C water bath such that the lip of the ampoule remains above the water line. Thawing time is approximately 2 to 3 minutes. Do not agitate the ampoule. Do not leave ampoule 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 2154 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



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overgrowth. 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™.



### 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<sub>2</sub>HPO<sub>4</sub> 1.0 g

KH<sub>2</sub>PO<sub>4</sub> 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.

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<sup>5</sup>/ml - 1 x 10<sup>6</sup>/ml using fresh medium. If the cell concentration is below 5 x 10<sup>5</sup>/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 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 2154 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.



### References

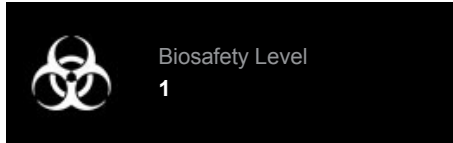
References and other information relating to this product are available online at [www.atcc.org](http://www.atcc.org).



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## **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.

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Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).

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