



Product Sheet

Entamoeba dispar (ATCC® PRA-260™)

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 dispar* (ATCC® PRA-260™)

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

Or contact your local distributor

Description

Strain Designation: SAW 760

Deposited Name: *Entamoeba dispar* Brumpt

Depositor: CG Clark

Isolation: Monoxenic strain established at NIH, Bethesda, MD in 1994; derived from a bacterized strain isolated from adult human male, England, 1979; the bacterized strain was obtained from PG Sargeant, London School of Tropical Medicine and Hygiene.

Propagation

Growth Conditions

Temperature: 35°C

Atmosphere: Microaerophilic

Culture System: Monoxenic with *Crithidia fasciculata* (ATCC 50083)

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.

Protocols

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 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 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.



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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

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
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 10⁵/mL - 1 x 10⁶/mL using fresh medium. If the cell concentration is below 5 x 10⁵/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 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



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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

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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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