ATCC

Product Sheet

Entamoeba dispar (ATCC® PRA-368™)

Please read this FIRST

Storage Temp.
Frozen: -70°C or colder
Freeze-Dried: 2°C to 8°C
Live Culture: See Protocols Section

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-368™)

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: NIH:0581:1
Deposited Name: *Entamoeba histolytica* Schaudinn
Depositor: LS Diamond
Isolation: Human

Notes

This xenic culture contains the original bacterial flora present when the amoeba was first isolated.

Propagation

Growth Conditions
Temperature: 35°C
Atmosphere: Microaerophilic
Culture System: Xenic

Medium
ATCC® Medium 1171: TYGM-9 medium

Instructions for Complete Medium
Grown with mixed bacteria. Quality controlled lots of this medium are commercially available from ATCC as cat. no. IV-1171.

Protocols

Storage and Culture Initiation
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. One day before thawing a frozen ampule inoculate two tubes of ATCC medium 1171 with the bacterial flora only. Incubate the tubes on a 15° horizontal slant at 35°C. If the specific bacterial flora associated with this culture are not available, skip this step and proceed to step 2.
2. On the following day combine 4.1 mL ATCC medium 1171 with 0.9 mL of HIBS (heat-inactivated bovine serum) to produce 5 mL of medium enriched with 20% serum. Invert gently several times to mix. **Note:** If bacterized medium 1171 was prepared in step 1, use the bacterized medium in place of fresh medium for faster recovery/growth.
3. Remove the frozen ampule from liquid nitrogen and flame gently at the base of the cap. Remove the cap and aseptically add 0.5 mL of the serum-enriched medium prepared in step 2. Place in a 35°C water bath until thawed (2-3 min). **Note:** Manipulations of the ampule before placing in the water bath should be done as quickly as possible to avoid warming of the contents at a suboptimal rate.
4. Transfer contents of the thawed ampule to a one-dram screw-capped vial (vial holds approximately 4.0 mL).
5. Add 2.5 mL of serum-enriched medium prepared in step 2 to the vial in dropwise fashion. Tighten the cap and incubate on a 15° horizontal slant at 35°C for 2-3 hours.
6. Ice the vial for 10 minutes, then invert gently 10 times. Centrifuge the vial at 100-200 x g for 5 min.
7. Aspirate the supernatant leaving approximately 0.5 mL. **Note:** Do not aspirate the pelleted material.
8. Replace the supernatant with 3.0 mL of the bacterized medium 1171 prepared in step 1 if available; otherwise use fresh medium 1171 (i.e., not serum-enriched).
9. Incubate the vial on a 15° horizontal slant at 35°C with the cap screwed on tightly. Observe the culture daily and transfer when many trophozoites are observed (i.e., early stationary phase).

Culture Maintenance
In general, addition of penicillin G at 75 U/mL and streptomycin at 75 ug/ml to ATCC Medium 1171 may be necessary if the bacterial density in the culture is too high. Inoculate fresh tubes of media with bacteria at least one day prior to subcultivation and prior to addition of antibiotics, if used.

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 1171.
2. Screw cap on tightly and incubate on a 15° horizontal slant at 35°C. Transfer when many trophozoites are observed (i.e., early stationary phase).

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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
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.

Harvest and Preservation

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.
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 x 10^5/mL - 1 x 10^6/mL using fresh medium. If the cell concentration is below 5 x 10^6/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 vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through heat of fusion. At -40°C plunge ampules into liquid nitrogen.
10. Store ampules in a liquid nitrogen refrigerator until needed.
11. One day before thawing a frozen ampule inoculate two tubes of ATCC medium 1171 with the bacterial flora only. Incubate the tube on a 15° horizontal slant at 35°C.
12. On the following day combine 4.1 mL of the bacterized medium 1171 prepared in step 11 with 0.9 mL of HIBS (heat-inactivated bovine serum) to produce 5 mL of medium enriched with 20% serum. Invert gently several times to mix.
13. Remove the frozen ampule from liquid nitrogen and flame gently at the base of the cap. Remove the cap and aseptically add 0.5 mL of the serum-enriched medium prepared in step 12. Place in a 35°C water bath until thawed (2-3 min). **Note:** Manipulations of the ampule before placing in the water bath should be done as quickly as possible to avoid warming of the contents at a suboptimal rate.
14. Transfer contents of the thawed ampule to a one-dram screw-capped vial (vial holds approximately 4.0 mL).
15. Add 2.5 mL of serum-enriched medium prepared in step 12 to the vial in dropwise fashion. Tighten the

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cap and incubate on a 15° horizontal slant at 35°C for 2-3 hours.

16. Ice the vial for 10 minutes, then invert gently 10 times. Centrifuge the vial at 100-200 x g for 5 min.
17. Aspirate the supernatant leaving approximately 0.5 mL. **Note:** Do not aspirate the pelleted material.
18. Replace the supernatant with 3.0 mL of the bacterized medium 1171 prepared in step 11.
19. Incubate the vial on a 15° horizontal slant at 35°C with the cap screwed on tightly. Observe the culture daily and transfer when many trophozoites are observed (i.e., early stationary phase).

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

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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