ATCC

Product Sheet

Entamoeba histolytica (ATCC® 30459™)

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
2

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 histolytica* (ATCC® 30459™)

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: HM-1:IMSS [ABRM]
Deposited Name: *Entamoeba histolytica* Schaudinn
Depositor: LS Diamond
Isolation: Colonic biopsy of rectal ulcer from adult human male with amebic dysentery, Mexico City, Mexico, 1967

Propagation

Growth Conditions
Temperature: 35°C
Culture System: Axenic, anaerobic

Medium
ATCC® Medium 2463: LYI-S-2 medium, modified

Instructions for Complete Medium
ATCC Medium 2463 (ATCC medium PRA-2154 modified with an additional 5% heat-inactivated bovine serum may also be used; quality controlled freeze-dried lots of this medium are commercially available from ATCC).

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. 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 13 mL ATCC Medium 2463. Screw cap on tightly and incubate on a 15° horizontal slant at 35°C.

Culture Maintenance

1. Ice culture at or near peak density for 10 min.
2. Gently invert culture 20 times.
3. Aseptically transfer a 0.1 and 0.25 mL aliquot to freshly prepared (no older than 7-10 d) tubes of ATCC medium 2463.
4. Screw caps on tightly and incubate at a 15° horizontal slant at 35°C.
5. 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.

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

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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 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 13 mL of ATCC medium 2463.
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.

Biosafety Level: 2

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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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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