ATCC

Product Sheet

Entamoeba histolytica (ATCC® 30190™)

Please read this FIRST

Storage Temp.

Frozen Cultures:
-70°C for 1 week; liquid N_2 vapor for long term storage

Freeze-dried Cultures:
2-8°C

Live Cultures:
See Protocols section for handling information

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® 30190™)

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: HB-301:NIH
Deposited Name: *Entamoeba histolytica* Schaudinn
Depositor: LS Diamond
Isolation: Feces from adult human male with amebic dysentery, Burma, 1960

Propagation

Growth Conditions
Temperature: 35°C
Atmosphere: Anaerobic
Culture System: Axenic

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

Handling of Live Culture
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.

1. When the culture arrives remove it promptly from the shipping container. **Do not store the culture at refrigeration temperatures before handling.**
2. To assure viability, immediately incubate on a 15° horizontal slant at 35°C for at least three hours before observing the culture.
3. 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.
4. Aseptically transfer 0.5 and 0.1 mL aliquots to two 16 x 125 mm screw-capped test tubes each containing 13 mL of sterilized ATCC medium 2154. 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.

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 ml and 0.25 mL aliquot to freshly prepared (no older than 7 to 10 days) tubes of ATCC medium 2154.
4. Screw caps on tightly and incubate at a 15° horizontal slant at 35°C.
5. Subculture every 10 to 14 days.

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

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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 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 sufficient 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 2154.
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.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

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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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