Entamoeba histolytica Schaudinn

50548™

Description

Strain designation: HB-301:NIH CL-1-4
Deposited As: *Entamoeba histolytica* Schaudinn
Type strain: No

Storage Conditions

Product format: Test tube

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 2

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always

used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Medium:
ATCC Medium 2154: LYI Entamoeba medium
Instructions for complete medium: Media: ATCC Medium 1978

Alternate Media: ATCC Medium 1141 may also be used for cultivation, and is available freeze-dried from ATCC
Temperature: 35°C
Atmosphere: Anaerobic
Culture system: Axenic

Handling Procedures

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 7-10d) tubes of ATCC medium 1978.

4. Screw caps on tightly and incubate at a 15° horizontal slant at 35°C.

5. Subculture every 10-14 days.

Reagents for 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

Casein Digest Peptone (BBL)	40.0 g
Yeast Extract	20.0 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.

Cryopreservation:

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 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 1978.

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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Entamoeba histolytica* Schaudinn (ATCC 50548)

References

References and other information relating to this material are available at www.atcc.org.

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Revision

This information on this document was last updated on 2024-10-24

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