ATCC

Product Sheet

# *Entamoeba histolytica* (ATCC® 50549™)

**Please read this FIRST**

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® 50549™)

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 CL-1-5
**Deposited Name:** *Entamoeba histolytica* Schaudinn
**Depositor:** LS Diamond
**Isolation:**
strain HB-301:NIH (=ATCC 30190) monoxenized and cloned via microisolation, then re-axenized

## Propagation

**Growth Conditions**
**Temperature:** 35.0°C
Duration: axenic; anaerobic

**Medium**
ATCC® Medium 2154: LYI Entamoeba medium

**Instructions for Complete Medium**
ATCC Medium 1978
(ATCC medium 1141 may also be used for cultivation, and is available freeze-dried from ATCC. Contact sales for more information)

**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.

## 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.

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.

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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.

**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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**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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