Entamoeba histolytica Schaudinn

50527™

Description

Strain designation: HM-1:IMSS Clone 6
Deposited As: *Entamoeba histolytica* Schaudinn
Type strain: No

Storage Conditions

Product format: Test tube
Storage conditions: See handling procedure

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeu
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 editi
in Microbiological and Biomedical Laboratories (BMBL), U.S. Department of Health and Human Services. It is your r
understand the hazards associated with the material per your organization's policies and procedures as well a
applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials.
that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liq
and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas pha

in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless nece
that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid ni

Certificate of Analysis

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

Growth Conditions

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).

Temperature: 35°C
Atmosphere: Anaerobic
Culture system: Axenic

Handling Procedures

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 vo
suspension is approximately 15.5 ml. When the culture arrives remove it promptly from the shipping contain
the culture at refrigeration temperatures before handling. To assure viability, immediately incubate on
slant at 35°C for at least three hours before observing the culture. There should be numerous attached tro
numbers are low the culture may have been exposed to temperature extremes in transit. Regardless of
culture, ice the culture for 10 min. and gently invert 20 times. Aseptically transfer 0.5 and 0.1 ml aliquots to t

screw-capped test tubes each containing 13 ml of sterilized ATCC medium 2154. Incubate the pa
at a 15° horizontal slant with the caps on tightly at 35°C. See below for routine maintenance proced

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 m
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

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
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
10N NaOH (approximately 0.7 ml). Adjust final volume to 10 ml with distilled water and filter sterilize.
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.
   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. Re
   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. Mak
   determination of the cell density and adjust the concentration of the cells between 5 x 10^5/ml - 1 x 10^6
   medium. If the cell concentration is below 5 x 10^5/ml, centrifuge the cell suspension and resuspend the
   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 supe
   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 temperatu
   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
   The cooling cycle should be initiated no less than 15 and no more than 30 minutes after the addition of
   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 mi
    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 contai
    ATCC medium 1978.
13. Screw cap on tightly and incubate at a 15° horizontal slant at 35°C. Observe the culture daily and transf
    trophozoites are observed.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following ma
Schaudinn (ATCC 50527)

References

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

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Revision

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