



Product Sheet

Leishmania amazonensis (ATCC® 50159™)

Please read this **FIRST**

Storage Temp.
Frozen Cultures:
-70°C for 1 week;
liquid N₂ 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: *Leishmania amazonensis* (ATCC® 50159™)

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: IFLA/BR/67/PH8

Deposited Name: *Leishmania mexicana amazonensis*

Depositor: WRAIR

Isolation: Phlebotomine fly, *Lutzomyia flaviscutellata*, Belem, Para, Brazil, 1976



Propagation

Growth Conditions

Temperature: 20°C to 25°C (room temperature)

Atmosphere: Aerobic

Medium

ATCC® Medium 807: Brain heart infusion blood agar

ATCC® Medium 1011: Diphasic blood agar medium

ATCC® Medium 1012: Diphasic blood agar medium

ATCC® Medium 431: Trypanosome medium

ATCC® Medium 2736: M199, Modified Medium

Instructions for Complete Medium

Medium: ATCC medium 807 Brain heart infusion blood agar (10% rabbit blood, BHI overlay)

Alternate Media: ATCC medium 1011 Diphasic blood agar medium (30% rabbit blood, Locke's Soln. overlay), ATCC medium 1012 Diphasic blood agar medium (10% rabbit blood, Locke's Soln. overlay), ATCC medium 431 Trypanosome medium (Locke's Soln. overlay), ATCC Medium 2736 Modified M199

Medium Note: As an alternative to blood-agar media, pure broth ATCC Medium 2736 may be used. Many strains will readily adapt to broth cultivation in standard tissue culture flasks; for strains that do not, broth may be used in screw-capped tubes, incubated with caps tightened in an upright position to reduce the liquid surface area available for gas interchange. Note: Some strains may not grow equally well in alternative media.



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 screw-capped borosilicate glass test tube containing an appropriate blood-agar medium (i.e., ATCC Medium 807). Alternatively, inoculate 10 mL complete ATCC medium 2736 in a T-25 flask or screw-capped test tube.
3. Incubate culture tubes vertically at 20-25°C with the caps screwed on tightly; flask cultures should be incubated flat.

Culture Maintenance

1. When the culture has reached or is near peak density, gently mix the culture by aspirating with a sterile pipette and aseptically transfer 0.1-0.5 mL to a new culture vessel containing fresh medium (broth cultures generally require larger inocula than cultures grown in tubes of blood agar).
2. Incubate culture tubes vertically at 20-25°C with the caps screwed on tightly; flask cultures should be incubated flat.
3. Transfer the culture every 3-4 days as described in step 1. 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.

Note: Some fastidious strains may not proliferate well in older growth medium made with blood. If growth is poor, wash cells in an osmotically-balanced saline solution, centrifuge to concentrate as indicated below, and use growth media made with fresh blood.



Cryopreservation



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Reagents

Cryoprotective Solution

DMSO 1.0 mL

Fresh growth medium* 9.0 mL

*when blood-agar media is used, the liquid overlay can be used to prepare the cryoprotective solution

Harvest and Preservation

1. Harvest cells from a culture which is at or near peak density by centrifugation at 1,300 g for 5 min.
2. Adjust concentration of cells to 2×10^7 /mL in fresh medium.
3. While cells are centrifuging prepare a 10% (v/v) solution of sterile DMSO in fresh medium (broth or liquid overlay from blood agar). The DMSO solution when first prepared will warm up due to chemical heat. The solution should be allowed to return to room temperature prior to use.
4. Mix the cell preparation and the DMSO solution in equal portions. The final concentration will be 10^7 cells/mL and 5% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution before the freezing process is begun should be no more than 15 min.
5. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryovials (special plastic vials for cryopreservation).
6. Place the ampules in a Nalgene 1°C freezing apparatus. Place the apparatus at -80°C for 1.5 to 2 hours and then plunge ampules into liquid nitrogen. (The cooling rate in this apparatus is approximately -1°C/min.)
7. Store in either the vapor or liquid phase of a nitrogen refrigerator.
8. To thaw a frozen ampule, place it in a 35°C water bath such that the lip of the ampule remains above the water line. Thawing time is approximately 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after thawed.
9. Immediately after thawing, aseptically transfer contents to a screw-capped borosilicate glass test tube containing an appropriate blood-agar medium (i.e., ATCC Medium 807). Alternatively, inoculate 10 mL complete ATCC medium 2736 in a T-25 flask or screw-capped test tube.
10. Incubate culture tubes vertically at 20-25°C with the caps screwed on tightly; flask cultures should be incubated flat.
11. Maintain as described above.



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

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

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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




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