ATCC

Product Sheet

Leishmania major (ATCC® 30012™)

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: *Leishmania major* (ATCC® 30012™)

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: No designation
Deposited Name: *Leishmania tropica* (Wright) Luhe
Depositor: LS Diamond
Isolation: Human, Teheran, Iran, 1949

Propagation

Growth Conditions
Temperature: 25°C
Culture System: Axenic

Medium
ATCC® Medium 431: Trypanosome medium
ATCC® Medium 1011: Diphasic blood agar medium
ATCC® Medium 1012: Diphasic blood agar medium
ATCC® Medium 2736: M199, Modified Medium
ATCC® Medium 807: Brain heart infusion blood agar

Instructions for Complete Medium
Media: ATCC medium 431 Trypanosome medium

Alternate media: ATCC medium 1011 Diphasic blood agar medium (30% rabbit blood), ATCC medium 1012 Diphasic blood agar medium (10% rabbit blood), ATCC Medium 2736 Modified M199, ATCC medium 807 Brain heart infusion blood agar (10% rabbit blood, BHI overlay) (some strains may not grow equally well in alternative media)

Medium Note: As an alternative to blood-agar media, pure broth ATCC Medium 2736 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.

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 sufficient to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer contents to a screw-capped borosilicate test tube containing ATCC Medium 431, or alternatively, inoculate 10 mL complete ATCC medium 2736 in a screw-capped test tube. Incubate the tube vertically at 25°C with the cap screwed on tightly.

Culture Maintenance

1. When the culture has reached or is near peak density, invert tube 10 times and aseptically transfer a drop from a Pasteur pipette (0.05 mL) to another test tube of fresh medium.
2. Incubate the culture vertically at 25°C with the cap screwed on tightly.
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. If growth is poor, wash cells in an osmotically-balanced saline solution, centrifuge to concentrate as indicated below, and use freshly-made growth media.

Cryopreservation

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 x $10^{7/}$mL in fresh medium.

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3. While cells are centrifuging prepare a 10% (v/v) solution of sterile DMSO in fresh medium (broth). 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 cryules (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, do not leave in the water bath, aseptically transfer the contents of the ampule into a fresh tube of ATCC medium 431, or alternatively, inoculate 10 mL complete ATCC medium 2736 in a screw-capped test tube.
10. Incubate vertically at 25°C with the cap screwed on tightly.
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.

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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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