Leishmania major (Yakimoff and Schokhor) Bray et al.

50122™

Description

Strain designation: MHOM/IL/67/JERICHO II
Deposited As: *Leishmania major* (Yakimoff and Schokhor) Bray et al.
Type strain: No

Storage Conditions

Product format: Frozen
Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

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 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: Media: ATCC medium 807 Brain heart infusion blood agar (10% rabbit blood, BHI overlay), ATCC medium 1011 Diphasic blood agar medium (30% rabbit blood), ATCC medium 1012 Diphasic blood agar medium (10% rabbit blood), ATCC medium 431 Trypanosome medium, ATCC Medium 2736 Modified M199 (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. 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.
Temperature: 20-25°C
Atmosphere: Aerobic
Culture system: Axenic

Handling Procedures

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 -70ºC 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.

Reagents for cryopreservation: 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

Cryopreservation:

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.
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 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, 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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Leishmania major* (Yakimoff and Schokhor) Bray et al. (ATCC 50122)

References

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

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Revision

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