



# *Leishmania major* (Yakimoff and Schokhor) Bray et al.

PRA-309™

## Description

**Strain designation:** Seidman

**Type strain:** No

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## Storage Conditions

**Product format:** Frozen

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

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## 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.

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## 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.

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## Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.atcc.org](http://www.atcc.org).

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## Growth Conditions

### Medium:

ATCC Medium 2736: M199, Modified Medium

ATCC Medium 1011: Diphasic blood agar medium

ATCC Medium 1012: Diphasic blood agar medium

ATCC Medium 807: Brain heart infusion blood agar

### Instructions for complete medium:

**Media:** ATCC medium 2736 (hemin optional)

**Alternate Media:** ATCC medium 1011, ATCC medium 1012, ATCC medium 807 (some strains may not grow equally well in alternative media)

**Temperature:** 20-25°C

**Culture system:** Axenic

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## 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 ampoules may be stored at or below  $-70^{\circ}\text{C}$  for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally  $-20^{\circ}\text{C}$ ).** Storage of frozen material at this temperature will result in the death of the culture.

1. To thaw a frozen ampule, place in a  $35^{\circ}\text{C}$  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 T-25 tissue culture flask containing 10.0 ml ATCC medium 2736. Incubate at  $20\text{-}25^{\circ}\text{C}$  with the cap screwed on tightly.

### Culture maintenance:

1. Agitate a culture at or near peak density and aseptically transfer 0.1-0.2 ml to a fresh flask of ATCC medium 2736.
2. Incubate at  $20\text{-}25^{\circ}\text{C}$  with the cap screwed on tightly.
3. Transfer the culture every 7-14 days as described in steps 1-2. 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.

### Cryopreservation:

1. Harvest cells from a culture that is at or near peak density by centrifugation at  $\sim 800 \times g$  for 5 min. Pool the cell pellets into a single tube.
2. Adjust the concentration of cells to  $2.0 \times 10^7/\text{ml}$ . If the concentration is too low, centrifuge at  $\sim 800 \times g$  for 5 minutes and resuspend the cell pellet with a volume of supernatant to yield the desired concentration.
3. Prepare a 10% (v/v) sterile DMSO solution in fresh medium as follows: Add the required volume of DMSO to a glass screw-capped test tube and place on ice. Allow the DMSO to solidify. Add the required volume of refrigerated medium. Dissolve the DMSO by inverting several times. If the DMSO solution is not prepared on ice, an exothermic reaction will occur that may precipitate certain components of the medium.

4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be  $10^7$  and 5% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution to the start of the freezing process should be no less than 15 min and no longer than 60 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 vials in a controlled rate freezing unit. From room temperature cool at  $-1^{\circ}\text{C}/\text{min}$  to  $-40^{\circ}\text{C}$ . If freezing unit can compensate for the heat of fusion, maintain rate at  $-1\text{ C}/\text{min}$  through heat of fusion. At  $-40^{\circ}\text{C}$  plunge ampules into liquid nitrogen.
  7. The frozen preparations are stored in either the vapor or liquid phase of a nitrogen refrigerator.
  8. To establish a culture from the frozen state place an ampule in a water bath set at  $35^{\circ}\text{C}$ . Immerse the vial enough to cover only the frozen material. Do not agitate the vial.
  9. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and inoculate into 10.0 ml of fresh ATCC medium 2736.
  10. Incubate the tube at  $20\text{-}25^{\circ}\text{C}$  with the cap screwed on tightly.
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## 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 PRA-309)

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## References

References and other information relating to this material are available at [www.atcc.org](http://www.atcc.org).

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## Revision

This information on this document was last updated on 2022-12-24

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