




Product Sheet


# *Leishmania mexicana* (ATCC® 50156™)

Please read this **FIRST**



Storage Temp.  
**Frozen: -70°C or colder**  
**Freeze-Dried: 2°C to 8°C**  
**Live Culture: See Protocols Section**

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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 mexicana* (ATCC® 50156™)

American Type Culture Collection  
PO Box 1549  
Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

800.638.6597 or 703.365.2700  
Fax: 703.365.2750  
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## Description

**Strain Designation:** MNYC/BZ/62/M379

**Deposited Name:** *Leishmania mexicana mexicana* (Biagi) Garnham

**Depositor:** WRAIR

**Isolation:** Nyctomys sumichrasti, Cayo District, Belize, 1962.

## Propagation

### Growth Conditions

**Temperature:** 25°C

**Culture System:** Axenic

### Medium

ATCC® Medium 807: Brain heart infusion blood agar

## 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 it 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 807. 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, aseptically transfer a drop from a Pasteur pipette (0.05 mL) to another test tube containing fresh ATCC medium 807. Do not allow the culture to overgrow. The culture crashes soon after reaching peak density.
2. Incubate the culture vertically at 35°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.

## Cryopreservation

### Harvest and Preservation


1. Harvest cells from a culture that is at or near peak density. Aseptically transfer the broth overlay to a plastic centrifuge tube and adjust the concentration of cells to  $2 \times 10^7$ /mL in fresh medium (broth). If necessary, cells may be concentrated by centrifugation at 800 x g for 5 min.
2. Prepare a 10% (v/v) solution of sterile DMSO in fresh medium (broth).
  - a. Add 1.0 mL of DMSO to an ice cold 20 x 150 mm screw-capped test tube;
  - b. Place the tube on ice and allow the DMSO to solidify (~5 min) and then add 9.0 mL of ice cold medium;
  - c. Invert several times to dissolve the DMSO;
  - d. Allow to warm to room temperature.
3. Mix the cell preparation and the DMSO solution in equal portions. Thus, 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 no less than 15 min and no longer than 30 min.
4. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
5. Place the vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through the heat of fusion. At -40°C plunge into liquid nitrogen. Alternatively, place the vials 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.)
6. The frozen preparations should be stored in either the vapor or liquid phase of a nitrogen refrigerator. Frozen preparations stored below -130°C are stable indefinitely. Those stored at temperatures above



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- 130°C are progressively less stable as the storage temperature is elevated. Vials should not be stored above -55°C.
7. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the vial just to a level just above the surface of the frozen material. Do not agitate the vial.
  8. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and inoculate a 16 x 125 mm screw-capped test tube containing ATCC Medium 807.
  9. Incubate the culture at 25°C.



### References

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

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### Disclaimers

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Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).

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