



Product Sheet

# *Leishmania garnhami* (ATCC® 50136™)

Please read this **FIRST**



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 garnhami* (ATCC® 50136™)

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## Description

**Strain Designation:** MHOM/VE/76/JAP78

**Deposited Name:** *Leishmania mexicana garnhami*

**Depositor:** WRAIR

**Isolation:**

human, Andes Mountains, Venezuela, 1976

## Propagation

### Growth Conditions

**Temperature:** 25.0°C

Duration: axenic

**Protocol:** ATCCNO: 11745 SPEC: See general instructions for thawing and storage of frozen material before proceeding. Add thawed contents to a single 16 x 125 mm glass screw-capped test tube of the appropriate medium. Incubate the culture vertically with the cap screwed on tightly. It is essential to establish cultures initially in small volumes. Once established, the culture can be scaled up to larger volumes. Vigorously agitate the culture and aseptically transfer 0.1 ml of culture to a fresh tube of medium weekly.

### Medium

ATCC® Medium 807: Brain heart infusion blood agar

### Instructions for Complete Medium

ATCC Medium 807

### 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 containing fresh ATCC medium 807.
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

1. Harvest cells from cultures that are 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 overlay). If necessary, cells may be concentrated by centrifugation at  $800 \times g$  for 5 min.
2. Prepare a 10% (v/v) solution of sterile DMSO in fresh medium (broth). Cool on ice.
3. 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 solution to the start of the freezing process should be 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  $-1^\circ\text{C}/\text{min}$  to  $-40^\circ\text{C}$ . If the freezing unit can compensate for the heat of fusion, maintain rate at  $-1^\circ\text{C}/\text{min}$  through the heat of fusion. At  $-40^\circ\text{C}$  plunge into liquid nitrogen. Alternatively, place the vials in a Nalgene  $1^\circ\text{C}$  freezing apparatus. Place the apparatus at  $-80^\circ\text{C}$  for 1.5 to 2 hours and then plunge ampules into liquid nitrogen. (The cooling rate in this apparatus is approximately  $-1^\circ\text{C}/\text{min}$ .)
6. The frozen preparations should be stored in either the vapor or liquid phase of a nitrogen refrigerator. Frozen preparations stored below  $-130^\circ\text{C}$  are stable indefinitely. Those stored at temperatures above  $-130^\circ\text{C}$  are progressively less stable as the storage temperature is elevated. Vials should not be stored above  $-70^\circ\text{C}$ .
7. To establish a culture from the frozen state place an ampule in a water bath set at  $35^\circ\text{C}$ . 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 vertically at  $25^\circ\text{C}$ . Observe the culture daily and transfer when numerous trophozoites are observed.

## References



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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.

### **ATCC Warranty**

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### **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](http://www.atcc.org)

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

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