**Strain Designation:** F-53  
**Deposited Name:** Hartmannella limax (Dujardin)  
**Depositor:** JL Griffin  

### Growth Conditions

**Temperature:** 25.0°C  
Duration: grown with Enterobacter aerogenes ATCC 13048 and with another hartmannellid

**Protocol:** ATCCNO: 30939 SPEC: Aseptically add 1 ml of sterile distilled water to the inner vial, remove the filter paper with a pair of forceps, and place it in the center of an agar plate of medium 997. Add the liquid remaining in the vial to the plate and spread it evenly over the surface of the plate. Incubate the plate at 25C. Trophozoites (amebae) should be evident within 2-3 days.

### Medium

**ATCC® Medium 997:** Fresh water ameba medium

### Instructions for Complete Medium

ATCC medium 997 optionally inoculated with **Klebsiella pneumoniae** subsp. pneumoniae (ATCC® 700831) or **Enterobacter aerogenes** (ATCC® 13048).

### Culture Maintenance

1. Streak an ATCC medium 997 plate with **Klebsiella pneumoniae** subsp. pneumoniae (ATCC® 700831) or **Enterobacter aerogenes** (ATCC® 13048) and incubate at 35°C overnight.
2. Remove an agar block (~5 mm²) with trophozoites from the edge of an agar plate culture and invert the block at the edge of the freshly bacterized plate.
3. Wrap the entire edge of the plate with parafilm and incubate upright at 25°C.
4. Repeat steps 1-3 at 10-14 day intervals.

**NOTE:** A monoxenic amoeba culture can be established in this manner using any suitable bacterial food source.

### Cryopreservation

**Cryoprotective Solution**

- **DMSO** 1.5 ml
- Dryl's solution (or similar) 8.5 ml
1. Mix the components in the order listed. When the medium is added to the DMSO the solution will warm up due to chemical heat.
2. Harvest cells from a culture which is at or near peak density by adding 5 ml ATCC medium 5080 (Dryl's solution) and washing cells into suspension. Rub the surface of the plate with a spread bar to detach adhering trophozoites.
3. Adjust the concentration of cells to at least 2 x 10⁹/ml in fresh medium.
4. Mix the cell preparation and the cryoprotective solution in equal portions.
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°C/min to -40°C. If freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through heat of fusion. At -40°C plunge ampules 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.)
7. Ampules are stored in either the vapor or liquid phase of a nitrogen refrigerator.
8. To establish a culture from the frozen state place the vial in a 35°C water bath. Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial. Immediately after thawing, aseptically transfer the contents of the ampule to the center of a fresh plate of ATCC medium 997. Distribute the material evenly over the plate using a spread bar.
9. Wrap the entire edge of the plate with parafilm and incubate upright at 25°C. Follow the protocol for maintenance of culture.
References

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

Biosafety Level: 1

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.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

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).

© ATCC 2013. All rights reserved. ATCC is a registered trademark of the American Type Culture Collection. [02/06]