**Acanthamoeba castellanii**  
(ATCC® 30011™)

**Deposited Name:** Acanthamoeba castellanii (Douglas) Page  
**Depositor:** A Castellani  
**Isolation:** Yeast culture, London, 1930

This culture contains *Escherichia coli*, used as the bacterial food source at time of deposit. Good growth of both the amoeba and bacterial food source may be possible without addition of other bacteria to the culture, though initial growth of the amoebae may be slower when culture is first established.

**Growth Conditions**

- **Temperature:** 25°C  
- **Culture System:** Xenic

**Medium**

- ATCC® Medium 997: Fresh water ameba medium  
- ATCC® Medium 711: PYB

**Instructions for Complete Medium**

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

**Alternate media:** ATCC medium 711 optionally inoculated with bacteria as above. (Note that ATCC medium 711 is a richer formula than ATCC medium 997 and will produce denser and faster bacterial growth. Excess bacterial growth can inhibit growth of amoebae.)

**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°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 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.
2. Immediately after thawing, aseptically transfer contents to a plate of ATCC medium 997. Distribute the material evenly over the plate using a spread bar.
3. Wrap the entire edge of the plate with parafilm and incubate upright at 25°C. Trophozoites should be seen within 5-7 d.

**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 d intervals.

**Cryopreservation**

**Reagents**

- **Cryoprotective Solution**  
- DMSO, 1.5 mL  
- Dryl's solution (or similar), 8.5 mL
Harvest and Preservation

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 (Dry'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 10^6/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 from the U.S.
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 refrigerat
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.

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.

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