Strain Designation: CCAP 1567/1 [229]
Deposited Name: Protacanthamoeba caledonica
Depositor: CCAP
Isolation: Morar Estuary, Scotland, 1977

### Growth Conditions
**Temperature:** 25.0°C

**Duration:** grown with Escherichia coli

**Medium**

ATCC® Medium 997: Fresh water ameba medium

### Instructions for Complete Medium

ATCC medium 997 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 d intervals.

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

### Cryopreservation

#### Cryoprotective Solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
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<tbody>
<tr>
<td>DMSO</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>Dryl's solution</td>
<td>8.5 ml</td>
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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 10⁶/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. Immers[...]
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

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