Acanthamoeba castellanii (Douglas) Page

30868™

Description

Acanthamoeba castellanii strain CCAP 1501/2g was isolated in 1974 from a human cornea in England.

Strain designation: CCAP 1501/2g
Deposited As: *Acanthamoeba castellanii* (Douglas) Page
Type strain: No

Storage Conditions

Product format: Frozen
Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 2

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local

or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Medium:
ATCC Medium 997: Fresh water ameba medium
ATCC Medium 711: PYB
Instructions for complete medium: ATCC Medium 997 or 711, optionally inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC 700831) or *Enterobacter aerogenes* (ATCC 13048)
Temperature: 25°C
Culture system: Xenic

Handling Procedures

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 or 711. 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 days.

Culture maintenance:

1. Streak an ATCC medium 997 or 711 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.

Reagents for cryopreservation: Cryoprotective Solution

DMSO, 1.5 mL

ATCC medium 5080 Dryl's solution (or similar), 8.5 mL

Cryopreservation:

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 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 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 or 711. 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.

Notes

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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Acanthamoeba castellanii* (Douglas) Page (ATCC 30868)

References

References and other information relating to this material are available at www.atcc.org.

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Revision

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30868

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