Please read this FIRST

Storage Temp.
**Frozen Cultures:**
-70°C for 1 week; liquid N₂ vapor for long term storage

**Freeze-dried Cultures:**
2-8°C

**Live Cultures:**
See Protocols section for handling information

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: *Acanthamoeba polyphaga* (ATCC® 30461™)

Description

**Strain Designation:** eye
**Deposited Name:** Acanthamoeba polyphaga (Puschkarew) Page
**Depositor:** GS Visvesvara
**Isolation:** Human corneal scrapings, Houston, TX, 1973

Growth Conditions
**Temperature:** 25°C
**Culture System:** Axenic

Medium
ATCC® Medium 712: PYG w/ Additives

Instructions for Complete Medium
ATCC Medium 712

Protocols

**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 tip 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 T-25 tissue culture flask or 16 x 125 mm plastic test tube containing 5 mL ATCC Medium 712.
3. Screw the cap on tightly and incubate the tube or flask at 25°C.

**Culture Maintenance**

1. When the culture is at or near peak density, vigorously agitate the culture.
2. Transfer approximately 0.25 mL to a fresh tube or flask containing 5 mL of fresh ATCC medium 712.
3. Screw the cap on tightly and incubate at 25°C (incubate test tubes at a 15° horizontal slant).
4. The amoebae will form an almost continuous sheet of cells on the bottom surface of the flask or test tube. Repeat steps 1 through 3 at 10 to 14 day intervals.

**Cryopreservation**

**Harvest and Preservation**

1. To achieve the best results set up cultures with several different inocula (e.g. 0.25 mL, 0.5 mL, 1.0 mL). Harvest cultures and pool when the culture that received the lowest inoculum is at or near peak density.
2. If the cell concentration exceeds the required level do not centrifuge, but adjust the concentration to between 2 x 10⁶ and 2 x 10⁷ cysts/mL with fresh medium. If the concentration is too low, centrifuge at 600 g for 5 min and resuspend the pellet in the volume of fresh medium required to yield the desired concentration.
3. While cells are centrifuging prepare a 15% (v/v) solution of sterile DMSO as follows: Add the required volume of DMSO to a glass screw-capped test tube and place it in an ice bath. Allow the DMSO to solidify. Add the required volume of refrigerated medium. Dissolve the DMSO by inverting the tube several times.
   **Note:** If the DMSO solution is not prepared on ice, an exothermic reaction will occur that may precipitate certain components of the medium.
4. Mix the cell preparation and DMSO in equal portions. Thus, the final concentration will be between 10⁶ and 10⁷ cells/mL and 7.5% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution before the freezing process is begun should be no less than 15 min and no longer than 30 min.
5. Dispense in 0.5 mL aliquots into 1.0 mL to 2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation).

6. Place the vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through the heat of fusion. At -40°C plunge into liquid nitrogen. (The cooling rate in this apparatus is approximately -1°C/min.)

7. The frozen preparations are stored in either the vapor or liquid phase of a nitrogen freezer.

8. To establish a culture from the frozen state, place an ampule in a water bath set at 35°C (2 to 3 min). Immerse the vial just sufficient to cover the frozen material. Do not agitate the vial.

9. Immediately after thawing, aseptically remove the contents of the ampule and inoculate into 5 mL of fresh ATCC medium 712 in a T-25 tissue culture flask or plastic 16 x 125 mm screw-capped test tube. Incubate at 25°C.

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

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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Disclaimers

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

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Additional information on this culture is available on the ATCC web site at www.atcc.org.

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