**Product Sheet**

**Chlorogonium elongatum**  
(ATCC® 50936™)

**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 ampules 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 in a 35°C water bath, until thawed (2–3 min). Immerse the ampule just sufficiently to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer the entire contents to a single 16 x 125 mm screw-capped test tube containing 5 mL of ATCC Medium 5 broth (or alternatively, 5 mL ATCC Medium 351 supplemented with 0.1% Na Acetate). Allow the tube to stand upright for one to several hours (or centrifuge very lightly for 3–5 min) to allow most cells to settle to the tube base.
3. Gently remove most of the supernatant (save in a secondary tube), then resuspend the remaining cells in additional fresh medium to a total volume of 5-6 mL.
4. Incubate tubes on a 15° horizontal slant with the cap screwed on loosely (loosened one half turn) at 20-25°C under a 14 hour light (~50 µEinsteins/m²/s irradiance)/10 hour dark cycle. **Note:** Some algae may grow poorly or not at all when recovered from the frozen state in broth media. In such cases, the algae should initially be recovered on agar media before eventually transferring to broth culture.

**Culture Maintenance**

1. Inoculate a tube of fresh broth medium with 0.25 mL from a growing culture at or near peak density.
2. Incubate tubes on a 15° horizontal slant with the cap screwed on loosely (loosened one half turn) at 20-25°C under a 14 hour light (~50 µEinsteins/m²/s irradiance)/10 hour dark cycle.
3. Subculture as necessary (i.e., typically every 14-21 days in broth media).

**Cryopreservation**

1. Harvest cells from a culture that is at or near peak density by centrifugation at 400-500 x g for 5 min.
2. Adjust the concentration of cells to 2 x 10⁶ - 2 x 10⁷/mL in fresh medium.
3. While cells are centrifuging prepare a 10% (v/v) solution of sterile DMSO in fresh medium.
4. Mix the cell preparation and the 10% DMSO in equal portions. Thus, the final concentration will be 10⁸ - 10⁹ cells/mL and 5% (v/v) DMSO. The time from the mixing of the cell preparation and methanol stock solution to the beginning of the freezing process should be no less than 5 min and no greater than 15 min.
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 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. Alternatively, place the vials in a Nalgene 1°C freezing
The frozen preparations should be stored in either the vapor or liquid phase of a nitrogen refrigerator.

To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the ampule and add to a centrifuge tube containing 5 mL of ATCC Medium 5 broth (or alternatively, 5 mL ATCC Medium 351 supplemented with 0.1% Na Acetate). Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and resuspend the remaining cells in additional fresh medium to a total volume of 5-6 mL.

Incubate tubes on a 15°C horizontal slant with the cap screwed on loosely (loosened one half turn) at 20-25°C under a 14 hour light (~50 μEinsteins/m²/s irradiance)/10 hour dark cycle.

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.

ATCC Warranty

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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

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

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