**Crypthecodinium cohnii** (ATCC® 30772™)

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**Storage Temp.**
- Frozen: -70°C or colder
- Freeze-Dried: 2°C to 8°C
- Live Culture: See Protocols Section

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**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 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 it in a 35°C water bath, until thawed (2-3 min). Immerse the ampule just sufficient to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer the entire contents to a screw-capped borosilicate test tube containing 5 mL of ATCC Medium 460. Do not distribute the thawed material to a larger volume of medium. It is essential to first establish the culture in a small volume. Incubate the tube at 20°C to 25°C with the cap loosened one half turn. Subculture every 10-14d.

**Culture Maintenance**
1. Inoculate a tube of fresh medium with 0.1 mL from a growing culture at or near peak density.
2. Incubate the tube at 20°C to 25°C with the cap loosened one half turn. Subculture every 10-14d.

**Cryopreservation**

1. Harvest cells from cultures which are at or near peak density. Aseptically transfer cells to 15 mL plastic centrifuge tubes and centrifuge at ~150 x g for 5 min.
2. Adjust the concentration of cells to 2 x 10⁹/mL with fresh medium, then dilute to half this concentration by adding an equal amount of a 15% (v/v) sterile glycerol solution in fresh medium.
3. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation). The time from mixing of the cell suspension and the glycerol solution, before the cooling cycle begins, should be no greater than 15 min.
4. 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.)
5. The frozen preparations should be stored in either the vapor or liquid phase of a nitrogen refrigerator. Frozen preparations stored below -130°C are stable indefinitely. Those stored at temperatures above -130°C are progressively less stable as the storage temperature is elevated. Vials can be stored between -80 and -70°C for no longer than one week.
6. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial.
7. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and transfer to a fresh tube containing 5 mL of ATCC Medium 460. Incubate the tube vertically at 20-25°C with the cap loosened one half turn. Subculture every 10-14d.

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**References**

References and other information relating to this product are available online at [www.atcc.org](http://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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