



Crypthecodinium cohnii **(Seligo) Javornicky**

30556™

Description

Strain designation: To (Tolo)

Deposited As: *Crypthecodinium cohnii* (Seligo) Javornicky

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 1

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 460: A2E6 medium

Instructions for complete medium: ATCC Medium 460

Temperature: 20-25°C

Culture system: Axenic

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 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 to 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 (or T25 flask) 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 (or flask) at 20°C to 25°C with the cap loosened one-half turn. Subculture every 10 to 14 days.

Culture maintenance:

1. Inoculate a tube (or T25 flask) of fresh medium with 0.1 ml from a growing culture at or near peak density.
2. Incubate the tube (or flask) at 20°C to 25°C with the cap loosened one-half turn. Subculture every 10 to 14 days.

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 2×10^6 /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 mL to 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 less than 15 min and no greater than 30 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°C 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 (or T25 flask) containing 5 mL of ATCC Medium 460. Incubate culture (if in a tube, incubate vertically) at 20°C to 25°C with the cap loosened one-half turn. Subculture every 10 to 14 days.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Crypthecodinium cohnii* (Seligo) Javornicky (ATCC 30556)

References

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

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Revision

This information on this document was last updated on 2025-03-17

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