ATCC

Product Sheet

Crypthecodinium cohnii (ATCC® 30812™)

Please read this FIRST

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

Biosafety Level
1

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: *Crypthecodinium cohnii* (ATCC® 30812™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Strain Designation: MB 3
Deposited Name: *Crypthecodinium cohnii* (Seligo) Javornicky
Depositor: CA Beam, M Himes
Isolation: *Sargassum* sp., Miami Beach, FL, 1977

Notes

Major Sibling Species Y

Propagation

Growth Conditions
Temperature: 20°C to 25°C
Culture System: Axenic

Medium
ATCC® Medium 460: A2E6 medium

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 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 (or a T-25 tissue culture 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 20-25°C with the cap loosened one-half turn. Subculture every 10-14d.

Culture Maintenance

1. Inoculate a tube (or T-25 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-25°C (test tubes are incubated upright with the cap loosened one-half turn). Subculture every 10-14 d.

Cryopreservation

Harvest and Preservation

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 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 - 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 stabile indefinitely. Those stored at temperatures above -130°C are progressively less stabile 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

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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 test tube (or T-25 flask) containing 5 mL of ATCC Medium 460. Incubate culture at 20-25°C (test tubes are incubated upright with the cap loosened one-half turn). Subculture every 10-14d.

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

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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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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