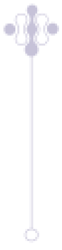

Cyanidioschyzon merolae P. De Luca, R. Taddei & L. Varano

PRA-402™

Description

Strain designation: 10D

Deposited As: *Cyanidioschyzon merolae*

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 2861: Acidic Allen Medium
Instructions for complete medium: Media: ATCC Medium 2861: Acidic Allen's medium

Alternate Media: ATCC Medium 616: Medium BG-11 for blue-green algae with pH adjusted to 2.3 with H_2SO_4.
Temperature: 42°C

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 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 until thawed (2-3 min). Immerse the ampule enough to cover only the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer contents to a 16 x 125 mm screw-capped test tube containing 5.0 mL of ATCC medium 2861. Incubate the tube on a 15° horizontal slant with the cap screwed on loosely (loosened one half turn) at 42°C under constant light.
3. Examine the culture using an inverted microscope for the presence of green microalgae. Growth of the strain may take several weeks to establish.

Culture maintenance:

1. Screw the cap on tightly and vigorously agitate the culture.
2. Aseptically transfer a 0.5 mL aliquot to 5 mL of fresh medium in a 16 x 125 mm screw-capped test tube.
3. Screw caps on loosely (loosened one-half turn) and incubate on a 15° horizontal slant at 42°C under constant light.
4. Subculture every 2-3 weeks.

Cryopreservation:

1. Harvest cells from a culture which is at or near peak density by centrifugation at ~800 x g for 10 min.
2. Adjust concentration of cells to 2 x 10^7 - 2 x 10^8 mL in fresh growth medium. If the concentration is too low, centrifuge at ~800 x g for 5 minutes and resuspend the cell pellet with a volume of supernatant to yield the desired cell concentration.
3. While cells are centrifuging, prepare a 10% (v/v) solution of sterile methanol in fresh growth medium.
4. Mix the cell suspension and the methanol solution in equal portions. The final concentration will be 10^7 – 10^8 cells/mL and 5% (v/v) of methanol. The time from the mixing of the cell preparation and methanol stock solution before the freezing process is begun should be no less than 5 min and no more than 15 min.
5. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryovials.
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 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.)

7. Store in either the vapor or liquid phase of a nitrogen refrigerator.
8. To thaw a frozen ampule, place it in a 35°C water bath such that the lip 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 it is thawed.
9. Remove the vial from the water bath immediately after thawing. Aseptically transfer the contents of the ampule into 5 mL of fresh ATCC medium 2861.
10. Incubate the tube on a 15° horizontal slant with the cap screwed on loosely (loosened one half turn) at 42°C under constant light.
11. Maintain as described above.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Cyanidioschyzon merolae* P. De Luca, R. Taddei & L. Varano (ATCC PRA-402)

References

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

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