Diplophrys marina Dykstra et Porter

PRA-22™

Description

Strain designation: CON-1
Deposited As: *Diplophrys marina* Dykstra et Porter
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 1728: Enriched Isonema medium
Instructions for complete medium: Note about growth media: This culture may grow equally well with serum concentration lowered to 5%.
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 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.
2. Immediately after thawing, aseptically transfer entire contents to a T-25 tissue culture flask containing 10 mL ATCC medium 1728. Incubate with the cap tightly sealed at 20-25°C.
   NOTE: Do not distribute the thawed material to a larger volume of medium than indicated above. It is essential to first establish the culture in a small volume.

Culture maintenance: Subculture every two weeks to a T-25 flask of fresh medium in the following manner:

1. Vigorously agitate the flask (or scrape the flask bottom using a sterile cell scraper) and aseptically transfer 0.2-0.3 mL from a growing culture to 10 mL of fresh ATCC medium 1728 in a T-25 tissue culture flask.
2. Incubate flask at 20-25°C with the cap on tightly.

Reagents for cryopreservation: Cryoprotective Solution

DMSO, 2.0 mL

Fresh growth medium, 8.0 mL

Cryopreservation:

Harvest and Preservation

1. Prepare a 20% (v/v) solution of sterile DMSO as follows: Add the required volume of DMSO to a glass screw-capped test tube and place it in an ice bath. Allow the DMSO to solidify. Add the required volume of refrigerated medium. Dissolve the DMSO by inverting the tube several times.
   *NOTE: If the DMSO solution is not prepared on ice, an exothermic reaction will occur that may precipitate certain components of the medium.
2. Harvest the culture by agitating the contents of each flask (or by scraping the bottom of each flask using a sterile cell scraper). Transfer the cell suspensions to 15 mL plastic centrifuge tubes.
3. Spin the cell suspensions at approximately 800 x g for 5 min.
4. Pool the cell pellets and adjust the concentration to 2.0 - 4.0 x 10^7 cells/mL with a fresh ATCC medium 1728.
   NOTE: If the concentration is too low, centrifuge at 500 x g for 5 min and

resuspend in the volume of ATCC medium 1728 required to yield the desired concentration.

5. Mix the cell preparation and 20% (v/v) DMSO in equal portions. The final concentration will be 1.0 - 2.0 x 10^7 cells/mL and 10% DMSO. The time from the mixing of the cell preparation and cryoprotective solution to the start of the freezing process should be no less than 15 min. and no more than 30 min.
6. Dispense in 0.5 mL aliquots to 1.0-2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
7. 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 ampules into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing apparatus. Place the apparatus at -80°C for 2 to 3 hours and then plunge ampules into liquid nitrogen. (The cooling rate in this apparatus is approximately -1°C/min.)
8. Store in either the vapor or liquid phase of a nitrogen refrigerator.
9. 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 thawed.
10. Immediately after thawing, aseptically transfer entire contents to a T-25 tissue culture flask containing 10 mL ATCC medium 1728. Incubate with the cap tightly sealed at 20-25°C.
11. Follow the protocol for maintenance of culture.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Diplophrys marina* Dykstra et Porter (ATCC PRA-22)

References

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

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Revision

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