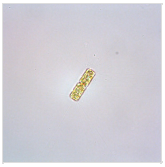

Aug 04, 2020

Cryopreservation of marine diatoms

Jana Hinners[1], Sinead Collins[1]

[1]University of Edinburgh

1 Works for me This protocol is published without a DOI.

Jana Hinners

ABSTRACT

Cryopreservation and thawing protocol for *Thallasiosira* diatoms.

ATTACHMENTS

Cryopreservation protocol.docx

PROTOCOL CITATION

Jana Hinners, Sinead Collins 2020. Cryopreservation of marine diatoms. **protocols.io** https://protocols.io/view/cryopreservation-of-marine-diatoms-bafqibmw

KEYWORDS

croypreservation, thawing, diatoms

CREATED

Dec 12, 2019

LAST MODIFIED

Aug 04, 2020

PROTOCOL INTEGER ID

30928

MATERIALS

NAME	CATALOG #	VENDOR
Liquid nitrogen		
Tissue Culture Flask, 50ml, 10/Cs	TCF70-50.SIZE.1PK	Bio Basic Inc.
DMSO	D1435	Sigma Aldrich
isopropyl alcohol	W292907	Sigma
Mr. Frosty Freezing Container, 2mL tubes, Nalgene Mr. Frosty Freezing Container for 1-2mL cryogenic tubes, PC, clear w/ blue lid, 1/Cs.	5100-0001	Thermo Fisher
Cryoboxes, 81, 2.0mL, gray	867013-0244	Thermo Fisher
Cryovial	V7884-450EA	Sigma Aldrich

SAFETY WARNINGS

Working with liquid nitrogen is dangerous and may require a special training.

Prepare diatom cultures 5d

1 Grow 40mL diatom cultures in 50mL flasks until mid to end exponential phase.
Do not invert samples for at least 24 hours before cryopreservation, so that diatoms are accumulated close to the bottom of the flask

1 day before cryopreservation 1d

2 Prepare fresh culture media (e.g. f/2) with 24% DMSO (=cryoprotective agent)
Depending on how dense the cultures are, the concentration of DMSO can be altered.
The final concentration of DMSO in the cryopreserved sample should be 12%.

3 Cool down Isopropyl alcohol (IPA) to 4 degrees Celsius

Cryopreservation day 1d

4 Concentrate the phytoplankton samples by removing the upper 30mL of each culture flask without disturbing the diatom biomass accumulated at the bottom

5 Fill Mr. Frosty with the 4 degree cold IPA.

6 Keep light intensity low for this step.

Mix concentrated phytoplankton sample 50:50 with the DMSO-media to obtain a 12% DMSO-concentration in the sample.

7 Keep light intensity low for this step.

Incubate samples for 15-30 min with DMSO-media.
In this time transfer replicates to cryovials, each receiving a volume of 1.6-1.8 mL depending on the chosen cryovial type.
Aim for at least 6 replicates per sample, since the survival rate of cryopreserved samples can vary.
I find it useful to handle 18 replicates at a time because they fit into 1 Mr Frosty.
Store the cryovials in the chilled Mr Frosty.

8 Store Mr. Frosty with cryovials at -80 degrees Celsius for at least 3-5 hours.
Do not close the Mr. Frosty too strong, otherwise it is hard to open the lid again.

9 After the 3-6 hours, remove the frozen cryovials from the Mr. Frosty, place them in a clearly labelled Cryobox (make sure to use a cryopservervation-safe label). Make sure you store the cryovials in a clear order and note down the order for long-term accessibility.
Store the cryobox in liquid nitrogen and note down the exact location of the box for long-term accessibility.
Success of the cryopreservation can be controlled after at least 24h in liquid nitrogen.

Thawing procedure 2d

10 Keep light intensity low for this step.

Thaw cryovials in a 37 degrees Celsius water bath until all water crystals disappeared (ca. 2 min).

11 Keep light intensity low for this step.

Dilute samples into 45 mL fresh media to dilute the DMSO.

12 Keep light intensity low for this step.

Place diluted sample into 4 degrees Celsius in the dark for 12 hours.

13 After 12h increase the temperature to standard growth temperature.

After another 12h increase the light intensity to standard light intensity.

14 Recovery and growth of cultures may take several days to weeks.