Fixation of marine samples for flow cytometry sorting

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Abstract

Protocol to fix marine samples for flow cytometry sorting of phytoplankton.
Fix at least 2 samples per depth sampled and at least 6 to 10 depths per vertical profile.

Back to the laboratory, pico and nano-plankton populations can be sorted and used for clone library construction or metabarcoding with Next Generation Sequencing (e.g. Illumina). This was successfully tested on samples from a cruise off Brazil (unpublished data)

Reference
Ribeiro C., Lopes A., Marie D., Vaulot D. Unpublished data

Citation: Daniel Vaulot Fixation of marine samples for flow cytometry sorting. **protocols.io**
dx.doi.org/10.17504/protocols.io.d2x8fm
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Before start

Necessary equipment
- Vortex mixer
- Cryotubes 2 mL
- Liquid nitrogen tank

Materials

Pluronic 10% P5556-100mL by Sigma Aldrich

DMSO 472301 by Sigma Aldrich

Protocol

Step 1.
Prefilter seawater sample onto 200 µm mesh

AMOUNT
2 ml Additional info:

Step 2.
Add 1.5 mL of prefiltered seawater sample to a 2 mL cryotube

Step 3.
Add DMSO

AMOUNT

150 µl Additional info:

REAGENTS

DMSO 472301 by Sigma Aldrich

Step 4.
Add Pluronic (facultative)

AMOUNT

2 µl Additional info:

REAGENTS

Pluronic 10% P5556-100mL by Sigma Aldrich

Step 5.
Vortex

Step 6.
Wait 10 min

DURATION

00:10:00

Step 7.
Flash freeze in liquid nitrogen tank

Warnings

Samples must be stored either in liquid nitrogen or at -80°C, not at -20°C because degradation will take place at the latter temperature