Fixation of marine samples for flow cytometry analysis

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Abstract

Protocol to fix marine samples for flow cytometry analysis of phtoplankton and bacteria with SYBR-Green.
Fix at least 2 samples per depth sampled and at least 6 to 10 depths per vertical profile

Reference
Marie, D., Rigaut-Jalabert, F. & Vaulot, D. (2014). An improved protocol for flow cytometry analysis of phytoplankton cultures and natural samples. *Cytometry*. 85. p.pp. 962–968.

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Before start

Necessary equipment

- Vortex mixer
- Cryotubes 2 mL
- Liquid nitrogen tank

Materials

Glutaraldehyde EM Grade 25% G5882-50ML by Sigma Aldrich

Pluronic 10% P5556-100mL 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 Glutaraldehyde

AMOUNT

15 µl Additional info:

REAGENTS

Glutaraldehyde EM Grade 25% G5882-50ML 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