Document Number: 001	Center for Aquatic Cytometry Bigelow Laboratory STANDARD OPERATING PROCEDURE	Category: Lab Work
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Author: Nicole Poulton	**Sample Fixation Procedure for Pico/Nanoplankton Flow Cytometry**	SOPs Referenced: none
Appendices: N/A		

1. PURPOSE

Preservation of pico/nanoplankton cells for rapid and accurate enumeration by flow cytometry. This method is derived from Vaulot et al., 1989.

2. DEFINITIONS/ACRONYMS

DIW	Deionized water
GF/F	Glass Fiber Filter
PBS	Phosphate Buffered Saline
FSW	0.2 m filtered seawater

3. APPARATUS AND REAGENTS

3.1. Paraformaldehyde powder
3.2. 1N NaOH (sodium hydroxide)
3.3. Stirring/hot plate
3.4. Chemical fume hood
3.5. pH meter
3.6. GF/F filter
3.7. Filtration apparatus
3.8. Cryovials (with O-ring)
3.9. Liquid N_2
3.10. Aluminum canes
3.11. 0.2 µm syringe filter
3.12. 100 µL pipette
3.13. 150-200 µm mesh nitex

4. SAFETY AND WASTE DISPOSAL

4.1. Chemical safety

4.1.1. The chemical paraformaldehyde is a flammable solid that is toxic if inhaled or ingested. It can cause skin irritation and serious damage to eyes. It is a suspected carcinogen. Use personal protective equipment (PPE) including safety glasses, gloves, lab coat and closed-toed shoes. Operate exclusively under a chemical fume hood. Read MSDS and understand all risks and safety precautions prior to handling.

4.1.2. Liquid N_2 is an extremely cold liquid and gas under pressure. Contact with eyes and skin can cause tissue freezing, cryogenic burns and frostbite. Inhalation can result in asphyxiation. Use PPE including face shield, insulated gloves, closed

toed shoes, and lab coat. Read MSDS and understand all risks and safety procedures prior to handling.

4.2. Hazardous chemical liquid and solid waste

4.2.1. Paraformaldehyde should be collected in a waste container under the chemical fume hood and neutralized prior to disposal.

4.2.2. Solid waste (e.g. cryovials and pipette tips) that have come in contact with paraformaldehyde should be collected in labeled zip-lock bags and brought to Chemical Storage.

5. PREPARATION

5.1. Paraformaldehyde (PFA) 10% solution

5.1.1. Materials

5.1.1.1. Paraformaldehyde powder
5.1.1.2. 1N NaOH
5.1.1.3. Stirring/hot plate
5.1.1.4. Chemical fume hood
5.1.1.5. pH meter
5.1.1.6. GF/F filters and filtration apparatus

5.1.2. Procedure

5.1.2.1. Mix 900 mL DIW and 100 g paraformaldehyde powder.
5.1.2.2. Place mixture on stirring hot plate under chemical fume hood.
5.1.2.3. Heat to approximately 60 ˚C (do not boil).
5.1.2.4. Stir for approximately 1 hour.
5.1.2.5. Turn off heat
5.1.2.6. Add 100 µL 1N NaOH to solution (Note: solution may not clear completely and you may need to add more NaOH in 10 µL increments until it is mostly clear).
5.1.2.7. Cool to room temperature.
5.1.2.8. Add 100 ml PBS or 0.2 µm FSW.
5.1.2.9. Filter through GF/F filter to remove precipitate.
5.1.2.10. Test pH (should be 7.4-8.0; approx. equal to seawater). If too acidic adjust with additional NaOH. Yields approx. 10% paraformaldehyde solution.

6. PROCEDURE

6.1. Pre-screening

6.1.1. Materials

6.1.1.1. 150-200 µm mesh nitex

6.1.2. Procedure

6.1.2.1. Examine sample for large particles (detritus, zooplankton).
6.1.2.2. If large particles are present, pre-screen sample through 150-200 µm mesh nitex.

6.2. Sample fixation procedure

6.2.1. Materials

6.2.1.1. Cryovials with O-rings
6.2.1.2. Liquid N_2
6.2.1.3. Aluminum cryostorage canes
6.2.1.4. 0.2 µm syringe filter

6.2.1.5. 10 or 20 mL syringe
6.2.1.6. Pipettors

6.2.2. Procedure

6.2.2.1. Filter paraformaldehyde through 0.2 µm syringe filter immediately before use, discarding the first few drops.
6.2.2.2. Add 50 µL of fresh filtered 10% paraformaldehyde to a labeled cryovial.
6.2.2.3. Add 1 mL of sample to cryovial. Samples should be labeled in permanent temperature resistant marker with an ID as well as the date (yyyy-mm-dd).
6.2.2.4. Cap and mix, turning vial end on end. Yields a 0.5% final concentration.
6.2.2.5. Place samples in refrigerator (4 ˚C) for 1-2 hrs to allow for fixation in the dark.
6.2.2.6. Place vials in labeled aluminum canes and put directly into liquid N_2.
6.2.2.7. Store samples in liquid N_2 or transfer to -80 ˚C freezer.

7. REFERENCES

Vaulot, D., Courties, C. & Partensky, F. 1989. A simple method to preserve oceanic phytoplankton for flow cytometric analyses. *Cytometry* 10:629-635.