Bigelow | Facility for Aquatic Cytometry

Nicole J. Poulton, Ph.D. - Director
Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

Sample Fixation Protocol for Pico/Nanoplankton Flow Cytometry

Preparation of Paraformaldehyde Solution

Materials
Paraformaldehyde powder
1N NaOH
stirring/hot plate
Chemical fume hood
pH meter
GF/F filters and filtration apparatus

1. Mix 900 mL DI water and 100 g paraformaldehyde powder.
2. Set up on a stirring/hot plate under hood.
3. Heat to approximately 60°C. Do not boil! Stir for approx 1hr.
4. Turn off heat.
5. Add 100 µL 1N NaOH to "clear" solution. NOTE: solution may not clear completely.
6. Cool to room temperature.
7. Add 100 mL phosphate buffer solution or filtered seawater.
8. Filter through GF/F filter to remove precipitate.
9. Test pH. Should be 7.4 - 8.0 (approx. equal to seawater). If necessary, add more NaOH. This yields a 10% solution (approximately).

Sample Fixation Procedure

Materials
cryovials with O-rings
liquid N_2
aluminum canes
0.2 µm syringe filter
10 or 20 mL syringe
pipettors

1. Filter paraformaldehyde through 0.2 µm syringe filter.
2. Add 50 µL of 10% paraformaldehyde to a labeled cryovial.
3. Add 1 mL sample to cryovial, cap, and mix. This yields a 0.5% final conc.
4. Allow fixation for 1 – 2 h in refrigerator (4°C) in the dark.
5. Place vials in labeled aluminum canes and put directly into liquid N_2.
6. Store samples in liquid N_2 – can be transferred to –80°C freezer.

Notes:
1. If sample has large particles (detritus, zooplankton) pre-screen through 150-200 um mesh nitex.
2. Cryovials can be pre-loaded with paraformaldehyde solution, then add sample, etc.
3. Method is derived from Vaulot et al. 1989

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