Document Number: 013	Center for Aquatic Cytometry Bigelow Laboratory STANDARD OPERATING PROCEDURE	Category: Lab Work
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Author: Nicole Poulton	**Preservation of Aquatic Samples Using Glutaraldehyde/Kolliphor**	SOPs Referenced: 0 see Marie et al 2014
Appendices: N/A		

1. PURPOSE

Preservation of prokaryotes, eukaryotes and pico/nanoplankton cells for flow cytometry. This method is derived from Marie et al 2014 with additional information provided by Dr. Heidi Sosik at Woods Hole Oceanographic Institution. The method was found to work well on diverse marine protists. Samples are preserved with a final concentration in sample of 0.125% glutaraldehyde and 0.01% Kolliphor P188.

2. DEFINITIONS/ACRONYMS

DIW Deionized water
EM Electron Microscopy
SDS Safety Data Sheet

3. APPARATUS AND REAGENTS

3.1. 25% EM grade glutaraldehyde
3.2. Kolliphor P188 (CAS No. 9003-11-6) **NOTE: This item is still available in large quantities through the manufacturer BASF, but is no longer available from Sigma Aldrich. A similar product Poloxamer 188 PRO product #P4894 is an alternative. Any generic poloxamer 188 is suitable as long it is high purity for cell culture use.**
3.3. Autoclave
3.4. Fume hood
3.5. -20 C freezer
3.6. 4 C refrigerator
3.7. Pipettors and tips (1000 μL and 20 μL)
3.8. 2 mL cryovials (with O-ring)
3.9. Liquid Nitrogen (Liquid N_2)
3.10. Aluminum cryocanes
3.11. 150 – 200 μm mesh (Nitex)

4. SAFETY AND WASTE DISPOSAL

4.1. Chemical safety and handling
4.1.1. **Glutaraldehyde is a highly toxic substance.** Wear personal protective equipment including gloves, eye protection, and lab coat and work in a fume hood. Avoid inhalation and contact with skin, eyes and clothing. Read SDS and understand all risks and safety procedures prior to handling. Note

that all waste that comes in contact with the glutaraldehyde (i.e. pipette tips for dispensing preservation mixture and contaminated gloves) must be disposed of using aldehyde waste procedures.

4.1.2. Liquid Nitrogen 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. Wear personal protective equipment including face shield, insulated gloves, closed toed shoes, and lab coat. Read SDS and understand all risks and safety procedures prior to handling.

5. PREPARATION

5.1. Kolliphor P188 2% Primary Stock Solution

5.1.1. Materials

5.1.1.1. DIW

5.1.2. Procedure

5.1.2.1. Dissolve 2 g of Kolliphor P188 in 80 mL of DIW.

5.1.2.2. Bring up to a total volume of 100 mL.

5.1.2.3. Autoclave to sterilize.

5.1.2.4. Kolliphor is not toxic and could be contaminated or grow bacteria. Store frozen at -20 C and defrost as needed to prepare the preservation mixture working stock. At the CAC we prefer to aliquot out 900uL aliquots into sterile cryovials. If not aliquoted, refilter through a 0.2um syringe filter immediately before preparation of working stock.

6. PROCEDURE

6.1. Prepare preservation mixture working stock (1:1 mixture of 2%kolliphor and 25% glutaraldehyde)

6.1.1. Materials

6.1.1.1. Kolliphor P188 primary stock solution, 2%

6.1.1.2. 25% EM grade glutaraldehyde

6.1.2. Procedure

6.1.2.1. Thaw a 900 μL aliquot of Kolliphor P188 2% primary stock solution.

6.1.2.2. Working in a fume hood, add 900 μL of 25% glutaraldehyde and mix well. Vortex if possible.

6.1.2.3. Store the preservation working stock at 4 C in the dark between uses and prepare a new working stock weekly.

6.2. Cryofixation procedure

6.2.1. Materials

6.2.1.1. Preservation mixture working stock (see 6.1)

6.2.1.2. Liquid N_2

6.2.2. Procedure

6.2.2.1. Examine samples for the presence of large particles (zooplankton, detritus, etc.)

6.2.2.2. If large particles are present, pre-filter the sample through 150-200 μm mesh to remove.

6.2.2.3. Add 1.8 mL of sample to cryovial. Vials should be prelabeled in permanent temperature resistant marker with an ID.
6.2.2.4. Working in a fume hood with the samples and preservative kept dark (you may cover them with aluminum foil), add 18 μL of the preservation mixture working stock.
6.2.2.5. Cap and gently mix, turning vial end on end.
6.2.2.6. Store at 4 C for 10 minutes.
6.2.2.7. Place the vials in labeled aluminum canes and put directly into liquid N_2 (flash freeze).
6.2.2.8. Store samples in liquid N_2 or transfer to -80 ˚C freezer for longer term storage.

7. ADDITIONAL NOTES

It is highly recommended that you prepare a preservative blank for your samples. This can be done by using 0.2 μm filtered seawater in place of the sample and preserving as normal.

The CAC also preserves flow cytometry samples using a paraformaldehyde solution (see SOP 001)

8. REFERENCES

Marie, D., Rigaut-Jalabert, F., & Vaulot, D. (2014). An improved protocol for flow cytometry analysis of phytoplankton cultures and natural samples. *Cytometry Part A*, *85*(11), 962-968.

9. VERSION UPDATES

2026-03-18 Updated chemical availability information.