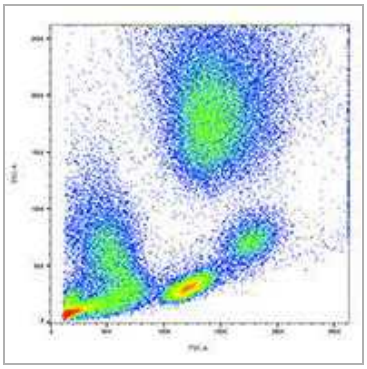

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Protocol status: Working
We use this protocol and it's working

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Water preservation for flow cytometry

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ABSTRACT

This method of preservation for flow cytometry was adapted from the TARA Oceans protocol for bacterial and viral flow cytometry.

This version of the protocol was edited by Rachel Cable for use in EXPORTS 2018 cruises and for field work in **Microbes in the Wild: Advanced Environmental Microbiology Lab (EEB 447)** course at University of Michigan Biological Station and on campus.

MATERIALS

MATERIALS

AQUEOUS GLUTARALDEHYDE 25% EM **Fisher Scientific Catalog #50-262-19**

Glutaraldehyde preparation

1 *For 1.5 mL sample volume:*

Aliquot 7.5 µL of 25% aqueous gluteraldehyde into each cryovial. Store aliquoted glutaraldehyde at 4°C until use.

AQUEOUS GLUTARALDEHYDE 25% EM **Fisher Scientific Catalog #50-262-19**

Note

We sample for FCM in triplicate, as most samples can only be completely defrosted and analyzed once.

Filtration preparation

2 Clean Swinnex filter holders by soaking in 10% bleach for 1-24 hrs. Then **rinse thoroughly** with MilliQ water. Allow to dry completely or use immediately. Syringes can also be bleached in the same manner and reused to reduce plastic waste.

Safety information

Please rinse filter holders until there is no hint of bleach smell and then an additional rinse or two. Any residual bleach will interfere with the flow cytometry of the samples. We learned this from the sampling in 2018 and lost the first 1-2 of each triplicate that past through the Swinnex after it was cleaned.

EMD Millipore Swinnex™ Filter Holders - Dia.: 25mm **Fisher Scientific Catalog #SX00 025 00**

3 Using sterilized forceps, place a 20 um nylon net filter in the filter holder and tightly screw the filter holder together.

20um Nylon Net Filter **Merck Millipore (EMD Millipore) Catalog #NY2002500**

Sample preservation

4 Using a sterile 10-ml syringe (see bleaching protocol in Step 2 if syringe is being reused) and the prepared filter cartridge, aliquot 1.5 mL of your 20 um-filtered seawater sample into each cryovial. Invert to mix. Label your cryovials with a ultra fine point Sharpie marker.

5 Incubate at room temperature in the dark for 15 minutes.

00:15:00 fixation

6 Snap-freeze the samples in liquid nitrogen. Store at -80°C.