Treatments for the Preservation of Seawater Samples

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Abstract

This protocol consists of several methods for the preservation of seawater samples to later perform various techniques in the lab. It is meant as a sampling treatment in the field and is written as comprehensive; however, depending on needs and scientific questions, any or all of the methods may be used.

Flow Cytometry (FCM): Samples are used for counting bacteria and for sorting different groups of microbes (eg, sorting synechococcus and prochlorococcus and non-fluorescent bacteria). Glutaraldehyde fixes microbes and renders them unculturable whereas microbes preserved in DMSO and Betaine can usually be cultured after thawing and rinsing. Betaine was developed for single cell sorting and may be a better preservative than DMSO.

SYBR staining: Samples are used for counting bacteria (unfiltered or pre-filtered seawater) and viruses (unfiltered, pre-filtered or 0.2um filtered seawater) microscopically after staining with SYBR-Gold (or SYBR-Green).

Transmission Electron Microscopy (TEM): Samples are used for determining frequency of visibly infected cells (FVIC) and determining types of microbes or viruses present, and can be quantitative.

Quantitative PCR (qPCR): Samples are used for quantitative PCR of microbes. They can also be used for quantitative PCR of viruses.

Viral qPCR: Samples are used for quantitative PCR of viruses.

Culturing Viruses: Samples are used for cultivating viruses by solid or liquid plaque assay on susceptible hosts. Samples can also be used for viral tagging (using flow cytometry) and most probable number (MPN) assays.

Fluorescence in situ hybridization (FISH): Samples are used with fluorescent probes to determine what populations of bacteria or other microbes are present. The filters may also be used to simply count the number of microbes in a sample. For detailed FISH procedure see here.

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Guidelines

***Pre-filter Seawater*:** This step is optional depending on sampling conditions and scientific questions to be answered.

1) Filter the seawater using a 10µm or 200µm nylon mesh or Nitex filter. Amounts needed for each protocol given below.

Flow Cytometry (FCM): Samples are used for counting bacteria and for sorting different groups of microbes (eg, sorting synechococcus and prochlorococcus and non-fluorescent bacteria). Glutaraldehyde fixes microbes and renders them unculturable whereas microbes preserved in DMSO and Betaine can usually be cultured after thawing and rinsing. Betaine was developed for single cell sorting and may be a better preservative than DMSO.

1) *Glutaraldehyde, 25%*:

- Requires 5ml of the 10µm or 200µm seawater filtrate.

a) Dispense 1ml filtrate into 1.2ml cryovial. Make 3 replicates.

b) Add 5µl of 25% glutaraldehyde to each (final 0.125%). Invert to mix.

c) Incubate dark for 15 min. Flash freeze in liquid nitrogen (can use nylon sock to submerge and retrieve samples). Store at -80°C .

2) *DMSO:*

- Requires 5ml of the 10µm or 200µm seawater filtrate.

a) Dispense 1ml filtrate into 1.2ml cryovial. Make 5 replicates.

b) Add 70µl of DMSO to each (final 7%). Invert to mix.

c) Flash freeze in liquid nitrogen (can use nylon sock to submerge and retrieve samples). Store at -80°C .

3) *Betaine:* (method from R. Stepanauskas, 10/17/09)

- Requires 5ml of unfiltered or 200µm filtered seawater.

a) Prepare betaine stock, 48%: Dissolve 48g betaine anhydrous in 80 ml MilliQ water, bring volume up to 100ml. Filter with 0.2µm PES filter. Store refrigerated. Re-filter every month.

b) Dispense 1ml unfiltered (or 200µm filtered) seawater into 1.2ml cryovial. Make 5 replicates.

c) Add 143µl prepared betaine stock. Invert to mix.

d) Flash freeze in liquid nitrogen (can use nylon sock to submerge and retrieve samples). Store at -80°C .

e) Betaine method notes:

- The method was found to work well on diverse marine and freshwater samples: both the numbers and the optical properties of prokaryote cells were well preserved and the downstream single cell FACS-MDA-16SPCR success rate was slightly better than for fresh samples.
- The method was found to work poorly on hypersaline samples (~350 psu): almost 50% cells were lost after the cryopreservation.
- The method was not tested for the preservation of protists.

SYBR staining: Samples are used for counting bacteria (unfiltered or pre-filtered seawater) and viruses (unfiltered, pre-filtered or 0.2um filtered seawater) microscopically after staining with SYBR-Gold (or SYBR-Green).

- Requires 12ml of unfiltered or 10µm or 200µm filtered seawater. If only viruses are to be examined, the pre-filtrate should be filtered through a 0.2µm PES filter to eliminate bacteria and other protists.

1) Dispense 3.92ml seawater into 5ml cryovial. Make 3 replicates.
2) Add 80µl 25% glutaraldehyde to each. Invert to mix.
3) Flash freeze in liquid nitrogen (can use nylon sock to submerge and retrieve samples). Store at -80°C .

Transmission Electron Microscopy (TEM): Samples are used for determining frequency of visibly infected cells (FVIC) and determining types of microbes or viruses present, and can be quantitative.

• Requires 12ml of unfiltered or 10µm or 200µm filtered seawater. If only viruses are to be examined, the pre-filtrate should be filtered through a 0.2µm PES filter to eliminate bacteria and other protists.

1) Dispense 3.92ml seawater into 5ml cryovial. Make 3 replicates.
2) Add 80µl 25% glutaraldehyde to each. Invert to mix.
3) Flash freeze in liquid nitrogen (can use nylon sock to submerge and retrieve samples). Store at -80°C .

Quantitative PCR (qPCR): Samples are used for quantitative PCR of microbes. They can also be used for quantitative PCR of viruses.

• Requires 150ml (coastal) or 300ml (open-ocean) filtrate for 3 qPCR filters.

1) Filter 50ml (coastal) or 100ml (open ocean) through 0.2µm nucleopore filter (25mm diameter). Note: Can save filtrates for Viral qPCR and Culturing Viruses preservation methods.
2) Pass 3ml TE buffer through filter to rinse.
3) Place filter membrane into 1.5ml screw-cap tubes with o-ring gasket.
4) Repeat to prepare 3 filters per sample.
5) Store at -80°C .

Viral qPCR: Samples are used for quantitative PCR of viruses.

• If 0.2µm filtrate from qPCR is not available, filter 330ml of 10µm or 200µm filtrate through 0.22µm Sterivex filters.

1) Filter 100ml 0.2µm filtrate though 0.02µm Anotop syringe filters (25mm diameter) into cleaned container.
2) Pass 10ml TM buffer through filter to rinse.
3) Label syringe filter (date, location & depth).
4) Wrap filter in parafilm (using a 4-square length), wrap in aluminum foil, label outside (date, location & depth) and put into ziploc bag with other filters from that depth profile.
5) Repeat to prepare 3 syringe filters per sample.
6) Store at -80°C.

Culturing Viruses: Samples are used for cultivating viruses by solid or liquid plaque assay on susceptible hosts. Samples can also be used for viral tagging (using flow cytometry) and most probable number (MPN) assays.

• If 0.2µm filtrate from qPCR is not available, filter 165ml of 10µm or 200µm filtrate through 0.22µm Sterivex filters.

1) Dispense 50ml 0.22µm filtrate into sterile 1.5 ml microcentrifuge tubes. Make 3 replicates.
2) Add 20µl chloroform (optional to keep down bacterial growth). Invert to mix.
3) Store at 4°C .

For detailed FISH procedure see here.

Materials: Listed here are the consumables needed for all methods. Please check individual methods for specific material needs and quantities.

- Seawater
- 10µm nylon mesh or Nitex screen
- 200µm nylon mesh or Nitex screen
- 0.2µm Nucleopore™ filter (25mm diameter) – Whatman 110606
- 0.2µm polyethersulfone (PES) filter
- 0.22µm Sterivex™ filters – Millipore

- 0.02µm Anotop® syringe filters (25mm diameter) – Whatman 6809-2102
- 0.45µm cellulose nitrate support filter (25mm diameter)
- 0.45µm cellulose nitrate support filter (47mm diameter)
- 0.2µm polycarbonate membrane filter (25mm diameter) – Millipore GTTP 025 00
- 0.2µm polycarbonate membrane filter (47mm diameter) – Millipore GTTP 047 00
- Glutaraldehyde, 25% (electron microscopy grade)
- DMSO (dimethylsulfoxide, tissue culture grade, sterile)
- Chloroform
- Betaine, anhydrous
- Formaldehyde (37%, fresh, unopened)
- Milli Q water
- TM buffer (1M Tris pH8, 0.45M NaCl, 0.1M $MgCl_2$)
- TE buffer (10mM Tris pH8, 1mM EDTA)
- 50ml centrifuge tubes
- 1.2ml cryovials
- 5ml cryovials
- 1.5ml screw-cap tubes with o-ring gasket
- Kimwipes
- Parafilm
- Ziplock bags
- Petri dishes (60mm) or PetriSlides™ Dish – Millipore PDMA04700
- Tube storage boxes

Materials

PetriSlides™ Dish PDMA04700 by Emd Millipore

Protocol