Treatments and Preservation of Seawater Samples for FISH

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Abstract

This protocol gives a method for treatment and preservation of seawater samples that will be subject to 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. Requires varying amounts of 10µm or 200µm filtrate (up to 11.5L for open ocean depth profile) as shown in Table I.

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Guidelines

Requires varying amounts of 10µm or 200µm filtrate (up to 11.5L for open ocean depth profile) as shown in Table I.

Table I. Samples from open ocean depth profile for FISH. Goal is for cell concentrations of about 10^6/ml.

Depth	Volume Seawater	Volume 37% Formaldehyde	# of Reps
10m	150ml	8ml	2
500m	1000ml	54ml	2
1000m	2000ml	108ml	2
2000m	2600ml	140ml	2
Total with reps	11.5 L	620ml	8 filters

Formaldehyde is not a stable reagent. For this reason, use a fresh, unopened bottle of 37% formaldehyde for fixing samples. Use the table above to determine how much reagent you will need.

Before start

Have fresh, unopened bottle of 37% formaldehyde on hand (see Table 1 to determine amount of reagent needed).

Materials

PetriSlides™ Dish PDMA04700 by Emd Millipore

Protocol

Cell Counts

Step 1.
Add formaldehyde at a final concentration of 1-2% to filtrate. Fix for 12-24 hr at 4°C.

Cell Counts

Step 2.
Setup frittered glass support (25mm diameter) in filtration manifold.

Cell Counts

Step 3.
Place a drop of Milli Q water (250-500µl) on support, float 0.45µm cellulose nitrate support filter (25mm diameter) on top, turn on vacuum (5 in of Hg) to lay filter flat with no air bubbles.

Cell Counts

Step 4.
Turn off vacuum and release pressure from chamber.

Cell Counts

Step 5.
Add another drop of Milli Q to support filter, float 0.2µm polycarbonate membrane filter (25mm diameter) on support filter (shiny side facing up!). Save filter separator sheets for storage later.

Cell Counts

Step 6.
Turn on vacuum (5 in of Hg) to lay filter flat with no air bubbles. Leave vacuum on.

Cell Counts

Step 7.
Place filter tower on membrane and clamp.

Cell Counts

Step 8.
Filter 1ml of fixed sample by applying gentle vacuum (5 inch Hg); filter 2 ml of the fixed sample onto a second filter. The support filter may be utilized for both samples.

Cell Counts

Step 9.
Remove filter from filter holder and put it on Kimwipes to dry. Cover, e.g. with the lid of a cryo box or a Petri dish. Allow to air-dry.

Cell Counts

Step 10.
Label membrane filter with pencil; place membrane filter between separator sheets (will prevent the membrane filters from sticking to each other or to the Petri dish).

Cell Counts

Step 11.
Seal Petri dish with parafilm, prevent dish from opening with tape, and put Petri dishes into a Ziploc bag

Cell Counts

Step 12.
Store at -20°C until processing. Filters can be stored frozen for several months without apparent loss of hybridization signal.

Samples for FISH

Step 13.

Setup frittered glass (47mm diameter) support in filtration manifold.

Samples for FISH

Step 14.

Place a drop of Milli Q water (1ml) on support, float 0.45µm cellulose nitrate support filter (47mm diameter) on top, turn on vacuum (5 in of Hg) to lay filter flat with no air bubbles.

Samples for FISH

Step 15.

Turn off vacuum and release pressure from chamber.

Samples for FISH

Step 16.

Add another drop of Milli Q to support filter, float 0.2µm polycarbonate membrane filter (47mm diameter) on support filter (shiny side facing up!). Save filter separator sheets for storage later.

Samples for FISH

Step 17.

Turn on vacuum (5 in of Hg) to lay filter flat with no air bubbles. Leave vacuum on.

Samples for FISH

Step 18.

Place filter tower on membrane and clamp.

Samples for FISH

Step 19.

Filter appropriate volume (see Table I) of fixed sample by applying gentle vacuum (5 inch Hg). The support filter may be utilized for several samples.

Samples for FISH

Step 20.

After complete sample filtration, wash filter and tower with 20-30 ml of sterile H2O; remove H2O by vacuum.

Samples for FISH

Step 21.

Remove filter from filter holder and put it on Kimwipes to dry. Cover, e.g. with the lid of a cryo box or a Petri dish. Allow to air-dry.

Samples for FISH

Step 22.

Label membrane filter with pencil; place membrane filter between separator sheets (will prevent the membrane filters from sticking to each other or to the Petri dish).

Samples for FISH

Step 23.

Seal Petri dish with parafilm, prevent dish from opening with tape, and put Petri dishes into a Ziploc bag

Samples for FISH

Step 24.

Store at -20°C until processing. Filters can be stored frozen for several months without apparent loss of hybridization signal.