Sampling for Flow Cytometry (FCM) Version 2

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Abstract

Collect and preserve samples for flow cytometry analysis. This protocol can be used for lab culture experiments or field samples.

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Materials

- P1000 micropipets and 1 ml filter tips by Contributed by users
- P200 micropipets and 200 µl filter tips by Contributed by users
- Labeled 1.2 ml cryo tubes by Contributed by users
- Repeator pipet and tips (that hold total volume of 100 µl and can dispense 2 µl) by Contributed by users
- Racks to hold cryo tubes by Contributed by users
- Cryo tube canes for dipping into liquid nitrogen by Contributed by users
- Labeled cryo boxes for -80°C storage by Contributed by users
- 25% Glutaraldehyde by Contributed by users
- Liquid Nitrogen: 1-3 L by Contributed by users
- artificial seawater salts by Contributed by users

Protocol

Step 1.

Advance preparation: for each sample to be collected, aliquot 900 µl of seawater (artificial seawater salts or complete sterile medium) into a labeled 1.2 ml cryovial.

NOTES
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Adjust the dilution if necessary for your samples (this protocol uses a 10-fold dilution; if your sample is very concentrated and/or you are extremely sample-limited, you can dilute more; if your sample is dilute (<1E6 cells/ml), then you might want to dilute less or not at all).

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If your samples are not seawater-based, substitute an appropriate diluent of the same ionic strength (e.g. sterile medium, sterile freshwater salts).

Step 2.

Collect sample: add 100 µl of sample into cryovial containing 900 µl of seawater. Keep cryovial cap clean and sterile.

Step 3.

Preserve/fix sample: In the chemical fume hood, lay out cryovial caps (inside cap facing up) and use the repeator pipet to add 5 µl of glutaraldehyde (25%) to each cap. Cap each tube and mix well by inverting a few times. Incubate for 10 minutes in the dark.

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Final concentration of glutaraldehyde is 0.125%.

Step 4.

Flash-freeze tubes in liquid nitrogen and store at -80°C until analysis.