Fixation of Planktonic Samples

Matthew Sullivan

Abstract

Modified after Glöckner et al. 1999

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Guidelines

Needed:

- PFA Fixative
- Water sample
- Formaldehyde (optional)
- Moistened support filter (0.45 µm pore size, cellulos nitrate, 47 mm diameter)
- Membrane filter (0.2 µm pore size, white polycarbonate (GTTP), 47 mm diameter)
- Filtration tower
- Vacuum
- Moistened support filter (0.45 µm pore size, cellulose nitrate, 25 mm diameter)
- Membrane filter (0.2 µm pore size, white polycarbonate (GTTP), 25 mm diameter)
- Sterile H2O
- Petri dish lid
- Parafilm

Protocol

Step 1.
Add freshly prepared PFA fixative *(see 'Fixing Cells with PFA Protocol')* to water sample to a final concentration of 1-2%.
Alternatively, use Formaldehyde, and fix for 12-24 hours at 4°C.

DURATION
12:00:00

Step 2.
Place a moistened support filter (0.45 µm pore size, cellulose nitrate, 47 mm diameter) **and a membrane filter** (0.2 µm pore size, white polycarbonate (GTTP), 47 mm diameter) **into a filtration tower**

Step 3.
Filter an appropriate volume of the fixed sample by applying gentle vacuum (5 inch)

NOTES

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Support filters may be utilized for several samples; for cell numbers of around 10^6 per ml, 10 ml of sample is generally sufficient

Step 4.
Place a moistened support filter (0.45 µm pore size, cellulose nitrate, 25 mm diameter; Sartorius, Germany) **and a membrane filter** (0.2 µm pore size, white polycarbonate (GTTP), 25 mm diameter; Millipore, Eschborn, Germany) **into a filtration tower**

Step 5.
Filter 1 ml of the fixed sample by applying gentle vacuum (5 inch)

AMOUNT
1 ml Additional info:

NOTES
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The support filter may be utilized for both samples

Step 6.
Filter 2 ml of the fixed sample on a second filter.

AMOUNT
2 ml Additional info:

NOTES
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The support filter may be utilized for both samples

Step 7.
After complete sample filtration, wash with 10-20 ml of sterile H_2O

AMOUNT
15 ml Additional info:

Step 8.
Remove H_2O by filtration

Step 9.
Put membrane filter on blotting paper for drying and cover, e.g. with the lid of a cryo box or a Petri dish.

NOTES
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If this is not available use kim wipes

Step 10.
Allow air-drying

Step 11.
Store each filter in a separate labelled Petri dish

Step 12.
Place membrane filter between separator sheets that the GTTP membrane filters were provided with

NOTES
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This will prevent the membrane filter from sticking to the Petri dish.

Step 13.
Seal Petri dish with parafilm

Step 14.
Store at -20°C until processing

NOTES
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Filters can be stored frozen for several months without apparent loss of hybridization signal.