PFA fixation and Percoll prep of sediments

Version 2

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ABSTRACT

Protocol for PFA fixation of sediment or culture samples and Percoll prep of sediments.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
2 mM Ca2 /5 mM K /7 mM glutamate solution		
2 N NaOH		
Paraformaldehyde powder (MW 90.1)		
10X PBS		
100% Ethanol		
1M TE buffer (1M Tris-HCl, 0.1M EDTA, pH 8.0)		
100 µM Pyrophosphate		

4% PFA in PBS

1 Heat 33 mL MQ water to 60°C in a 50 mL Falcon style tube.

33 ml **Milli-Q water**

2 In the fume hood, add 2 g paraformaldehyde powder (MW 90.1) and a few drops of 2 N NaOH (1-4).

2 g **paraformaldehyde powder (MW 90.1)**

3 Close and mix by gently shaking tube to dissolve. Add an additional drop of 2 N NaOH if this takes more than 5 minutes.

4 Add 15 ml 10X PBS to bring to yield 4% PFA in 3X PBS (best for seawater samples), or 5 ml 10X PBS to yield 4% PFA in 1X PBS (best for freshwater samples).

5 Cool solution to room temperature and bring the final volume to 50 mL with MQ water.

6 Sterilize through a 0.2 µm syringer filter, and the store in 2-5 ml aliquots at -20°.

7 Some precipitate will likely be observed as the aliquots thaw. Place them in a beaker of room temperature water and give 10-15 minutes, and invert tubes to fully resuspend the solution.

00:15:00 **room temperature water**

Fixing sediment or culture samples (lab based)

8 Transfer ~0.1 g of wet sediment, crushed carbonate, or 0.5 to 1 ml of a culture to a 2 ml centrifuge tube.

9 Centrifgue at 10000g for 2 min to pellet sample and decant the liquid.

00:02:00 **Centrifugation at 10000g**

10 If the sample was taken from a stable isotope incubation, before fixation wash three times with PBS by resuspending and centrifuging to pellet.

11 Fully resuspend sample in 250 µl 3X PBS. (Use an alternate PBS concentration if desired).

250 µl **3X PBS**

12 Add 750 µl 4% PFA, invert tube to mix, and then incubate at 4°C for 1-3h.
(Alternatively, resuspend sample in 500 µl 3X PBS, add 500 µl 4% PFA and incubate at 4°C overnight.)

750 µl **4% PFA**

03:00:00 **Incubation at 4°C**

13 Centrifuge at 10000-16000g for 3 min to pellet.

00:03:00 **Centrifugation at 10000-16000g**

14 Decant the supernatant, and wash by fully resuspending the sediment in any volume of 3X PBS.

15 Centrifuge at 10000-160000g for 3 min to pellet, and decant the supernatant.

00:03:00 **Centrifugatio at 10000-16000g**

16 Resuspend in 1:1 3X PBS and 100% ethanol. Add PBS first, then add a complementary volume of ethanol and mix well.

17 Store samples indefinitely at -20 °C.

Fixing sediment samples (shipboard)

18 Add 0.5 of sediment to a 2 ml screw top tube,

19 Bring the volume to 1 ml with 3X PBS. Invert to suspend sediment in a slurry.

20 Add 1 ml of 4% PFA and invert to mix well.

1 ml 4% PFA

21 Incubate at 4°C for 12 hours.

12:00:00 Incubation at 4°C

22 Centrifuge at 10000g for 2 min to pellet.

00:02:00 Centrifugation at 10000g

23 Decant the supernatant, and wash by fully resuspending the sediment in any volume of 3X PBS.

24 Centrifuge at 10000g for 3 min to pellet, and decant the supernatant.

00:03:00 Centrifugation at 10000g

25 Resuspend in 1:1 3X PBS and 100% ethanol. Add PBS first, then add a complementary volume of ethanol and mix well.

26 Store samples indefinitely at -20 °C.

NOTE
If a -20 °C is not available, store at 4 °C until returning to the lab.

Mini Percoll prep for microscopy

27 For 200 µl of sediment slurry, add 600 µl 1M TE buffer (1M Tris-HCl, 0.1M EDTA, pH 8.0) and 100 µl 100 µM pyrophosphate to a 2 ml tube.

600 µl 1M TE buffer (1M Tris-HCl, 0.1M EDTA, pH 8.0)

100 µl 100 µM pyrophosphate

28 Heat the mixture in the hybridization microwave (or heat block) at 60°C for 3 min.

00:03:00 microwave (or heat block) at 60°C

29 Allow the tubes to cool on ice, and then sonicate on ice for 10 sec at power setting 3 (Branson Sonifier 150 with serial#C4333 wand style probe). (1/3)

00:00:10 Sonicate on ice

30 Sonicate on ice for 10 sec at power setting 3 (Branson Sonifier 150 with serial#C4333 wand style probe). (2/3)

00:00:10 Sonicate on ice

31 Sonicate on ice for 10 sec at power setting 3 (Branson Sonifier 150 with serial#C4333 wand style probe). (3/3)

00:00:10 Sonicate on ice

32 Add, ~1 ml percoll (filter sterilized with a 0.2 µm syringe filter) to the bottom of the sediment slurry with a pipette or needle.

33 Centrifuge at maximum speed (~16,000g) for 20 min at 4°C.

00:20:00 Centrifugation at ~16,000g

NOTE
After 20 min, the mixture will be separated by density, with
the sediment in a pellet at the bottom and two distinct liquid layers. The bottom layer is primarily percoll and the top is primarily
TE/PBS/ethanol. At the interface, expect to see a brown, “fluffy” band of organic material including single cells and aggregates.

34 Remove the upper layer and the organic material, transferring to a new tube and leaving as much of the percoll behind as possible.

35 Concentrate the cell layer by centrifuging at maximum speed for 3 min.

00:03:00 Centrifugation at maximum speed

36 Remove the supernatant and resuspend in an appropriate volume of 1:1 3X PBS:100% Ethanol. Depending upon cell density 100-300 µl.

37 The mixture can now be stored at -20°C for subsequent FISH hybridization after spotting on slides or filter preparation.