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RNA extraction from Sterivex using phenol:chloroform

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ABSTRACT

This extraction protocol uses bead-beating techniques within the Sterivex to dislodge biomass from the Sterivex filter. Biomass in solution is then removed from the Sterivex and RNA is extracted using acid phenol and chloroform. The protocol has been tested and used on eutrophic freshwater samples dominated by toxic cyanobacteria (*Microcystis*).

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KEYWORDS

RNA extraction, Sterivex, aquatic systems, bead-beating

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GUIDELINES

All steps should be performed in a chemical fume hood.

MATERIALS TEXT

- Sterivex containing sample
- Refrigerated microcentrifuge
- 2 mL eppendorphs (nuclease free)
- 20% SDS
- 0.5M EDTA
- 3M sodium acetate
- Nuclease free water
- Zymo ZR BashingBead Lysis Tubes (S6012-50)
- Mini-bead beater
- Acid phenol:chloroform:IAA (125:24:1; pH 4.8)
- Pure chloroform
- 100% ethanol (ice cold)
- 70% ethanol (ice cold)
- Heating block at 37 °C

SAFETY WARNINGS

Please review MSDS for all materials. Carcinogenic and corossive materials are used.

BEFORE STARTING

Set centrifuge to 4° C and allow to pre-cool.
Bring acid phenol:chloroform out of the -20°C freezer and allow to thaw in chemical hood.

RNA extraction

1 Make solution A: 750 µL of 20% SDS, 600 µL of 0.5M EDTA, 200 µL of 3M sodium acetate, 28.45 mL of nuclease free water

1.1 Make fresh weekly.

2 Remove luer-lock cap from Sterivex and carefully pour beads from Bashing Bead Lysis tube into the Sterivex

2.1 This is challenging! A funnel helps if you can sterilize one. Save the BashingBead tube.

3 Add 700 µL of solution A to Sterivex.

3.1 Insert pipette past the neck of the Sterivex before ejecting liquid.

4 Replace luer-lock cap and vortex for 20 min. 20m

4.1 If Sterivex don't spin naturally on vortex, rotate the Sterivex 180° after 10 min and continue vortexing.

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5 Return Sterivex to ice, luer-lock side down to allow bubbles to dissipate.

6 Use pliers to squeeze around the end of the Sterivex (opposite from the luer-lock). The Sterivex should pop apart and you can remove the end with the internal filter attached.

7 Pipette all liquid from the Sterivex and put it back into the original Bashing Bead lysis tube (which should be sitting empty).

7.1 It's ok if you suck up some beads too.

8 Add 500 µL acid phenol:chloroform:IAA (125:24:1; pH 4.8) to the BashingBead tube.

8.1 You should be pulling from the bottom layer of the thawed phenol:chloroform mixture.

9 Beat on maximum speed for 40 s in bead beater and place on ice briefly to chill. 40s

10 Spin at maximum speed in a 4 °C benchtop centrifuge for 5 minutes. 5m

10.1 13,300 rpm for our centrifuge.

11 Pull off as much of the aqueous supernatant as possible from the beating tube and place in a new 2 mL centrifuge tube.

11.1 If there is a large white protein layer between your top and bottom layer, you may want to repeat the 500 µL acid phenol:chloroform:IAA addition, vortex, centrifuge, and add supernatant to new tube. You risk losing some RNA, but you can increase the quality. 5m

12 Add 500 µL chloroform to aqueous layer, vortex well, spin at max speed for 5 min. 5m

13 Repeat chloroform extraction to remove residual phenol. 5m

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Ethanol Precipitation

14 Add sodium acetate to ~0.3 M and mix.

14.1 For 500 µL recovery, add 50 µL of 3M NaAc.

15 Add 2-2.5x volumes of ice cold 100% ethanol and mix well.

15.1 Typically fill the rest of the 1.5 mL microcentrifuge tube with ethanol.

16 Place in -80 °C freezer for at least 1 hr. 1h

16.1 Can be stored at -80 °C overnight.

RNA pellet wash 45m

17 Spin at maximum speed at 0 °C for at least 30 min in refrigerated centrifuge. 30m

17.1 If you suspect low yields, spin for 1 hr at max.This seems to help in RNA (or DNA) recovery.

18 Carefully decant 100% ethanol without disturbing RNA (or DNA) pellet.

18.1 You may not be able to see your pellet so be careful to avoid where it would be located.

19 Wash pellet by adding 1 mL of ice cold 70% ethanol and gently pipetting up and down enough to dislodge pellet.

19.1 If you can see a pellet, you have at least ~2-5 µg nucleic acid. Do not panic if you cannot see pellet.

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20 Spin at maximum speed at 0 °C for 15 minutes. 15m

20.1 Spin for 30 minutes if you expect low yields.

21 Gently and carefully aspirate as much of the 70% ethanol from the tube as possible taking caution not to accidently aspirate the pellet OR use a small pipette tip to suck out as much ethanol as you can.

22 Place the open tube in a 37 °C heating block for about 3-5 min to evaporate all ethanol. 5m

22.1 Try to place heating block in sterile environemnt to avoid falling contamination, given that the tubes need to remain open during this step. It may take longer if you did not remove all excess ethanol in previous step.

23 Dissolve the RNA (or DNA) pellet in the appropriate amount of RNAse/DNase-free water depending on expected yields and needed final concentration.

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