RNA Miniprep using 2-Butoxyethanol

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Version: 1.0 - Print Version (.PDF)

ATTENTION: This is a low priced protocol. Use it preferably!
It works well, but it takes three days and involves many toxic chemicals.

1. Add 1% beta-mercaptoethanol to the extraction buffer
2. Grind max. 125 mg tissue into a fine powder
3. Transfer grinded powder into a eppi
4. Add **1 ml of extraction buffer** and vortex well
   Careful, pressure may build up!
5. Spin for **10 min** at top speed
6. Transfer top phase to a fresh eppi
7. Add **500 µl chloroform** and vortex well
8. Spin for **5 min** at top speed
9. Transfer top phase to a fresh eppi
10. Add **500 µl phenol / chloroform** and vortex well
11. Spin for **10 min** at top speed
12. Transfer top phase to a fresh eppi
13. Add **500 µl chloroform** and vortex well
14. Spin for **5 min** at top speed
15. Transfer top phase to a fresh eppi
16. Add **40 µl 3 M NaAc and 1 ml 96% EtOH** and vortex well
17. Store at -80 °C until frozen
18. Spin for **5 min** at top speed at **4 °C**
19. Remove supernatant and add **500 µl 70% EtOH** to wash
20. Spin for **5 min** at top speed
21. Remove supernatant and add **500 µl RNA resuspension buffer**
22. Incubate at 65 °C to dissolve pellet
23. Add **200 µl 2-Butoxyethanol** an mix well
24. Incubate on ice for at least 3 hours.
25. Spin for **10 min** at max. speed at **4 °C**
26. Transfer supernatant to a fresh eppi
27. Add **300 µl 2-Butoxyethanol** and mix well
28. Incubate on ice over night.

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29. Spin for **10 min** at max. speed at **4 °C**
30. Discard the supernatant and add **500 µl 70% EtOH**
31. Spin for **5 min** at max. speed at **4 °C**
32. Discard the supernatant and let pellet briefly dry
33. Dissolve in **1 ml water** (and transfer to an eppi)
34. Precipitate RNA by adding **335 µl 8 M LiCl**
35. Incubate at 4 °C over night
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36. Spin for **30 min** at max. speed at **4 °C**
37. Discard the supernatant and add **100 µl 70% EtOH**
38. Spin for **5 min** at max. speed
39. Discard the supernatant and dissolve in **200 µl water**
40. Add **20 µl 3 M NaAcetate (pH 5.2) and 500 µl 96% ethanol**
41. Spin for **5 min** at max. speed
42. Store at -80 °C until frozen
43. Spin for **30 min** at top speed at **4 °C**
44. Remove supernatant and add **100 µl 70% EtOH** to wash
45. Spin for **5 min** at top speed
46. Remove supernatant and redissolve **50 µl water**
47. Incubate at 65 °C to dissolve pellet

Buffers and Solutions

RNA Extraction Buffer

Prepare in a 1 liter bottle:

Triisopropylnaphtalene Sulfonic Acid	5.56 g	1%
p-Aminosalicylic Acid	30 g	6%
1 M Tris-HCl (pH 8.0)	50 ml	100 mM
0.5 M EGTA (pH 8.0)	50 ml	50 mM
NaCl	2.9 g	100 mM
SDS	5 g	1%
Polyvinylpyrolidone (PVP) (M.W. 40,000)	5 g	1%
Polyvinylpolypyrolidone (PVPP)	15 g	3%
add water to 500 ml		

Autoclave for 15 min, then add:

chloroform	500 ml	

The extraction buffer is a mess. The PVPP will not dissolve, the chloroform will always separate and it has a brown color. The best is to put it on a magnetic stirrer and use it from there. It has a cacao color and can be pipetted with 1 ml pipettes were the tip was cut off.

Always add beta-mercaptoethanol fresh.

Before use add Mercaptoethanol:

for 50 ml Buffer add 250 µl Mercaptoethanol
for 30 ml Buffer add 150 µl Mercaptoethanol
for 10 ml Buffer add 50 µl Mercaptoethanol
for 5 ml Buffer add 25 µl Mercaptoethanol
for 1 ml Buffer add 5 µl Mercaptoethanol

RNA Resuspension Buffer

Boric Acid	0.78 g	25 mM
1 M Tris-HCl (pH 7.6)	25 ml	50 mM
0.5 M EGTA (pH 8.0)	1.3 ml	1.25 mM
NaCl	2.9 g	100 mM
add water to 500 ml		

Required Solutions and Chemicals

- 3 M Natriumacetate (NaAc) solution (pH 5.2)
- 2-Butoxyethanol (2BE)
- 8 M Lithiumchloride (LiCl)

Commented Protocol:

1. Add 1% beta-mercaptoethanol to the extraction buffer

Because the extraction buffer always separates it is the best to put it on a magnetic stirrer and use it from there. It can be pipetted with 1 ml pipettes were the tip was cut off. Always add beta-mercaptoethanol fresh.

2. Grind max. 125 mg tissue into a fine powder

This is best done in a mortar for big amounts with liquid nitrogen or in a cap-shaker for smaller amounts.

3. Transfer grinded powder into a eppi

4. Add 1 ml of extraction buffer and vortex well

Careful, pressure may build up!

5. Spin for 10 min at top speed

The chloroform is already in the extraction buffer.

6. Transfer top phase to a fresh eppi

7. Add 500 µl chloroform and vortex well

8. Spin for 5 min at top speed

9. Transfer top phase to a fresh eppi

10. Add 500 µl phenol / chloroform and vortex well
Just mix phenol and chloroform 1:1 and mix well before use. It also separates after some time.

11. Spin for 10 min at top speed

12. Transfer top phase to a fresh eppi

13. Add 500 µl chloroform and vortex well

14. Spin for 5 min at top speed

15. Transfer top phase to a fresh eppi

16. Add 40 µl 3 M NaAc and 1 ml 96% EtOH and vortex well
This step precipitates all nucleic acids. The pellet has a brown color.

17. Store at -80 °C until frozen

18. Spin for 5 min at top speed at 4 °C

19. Remove supernatant and add 500 µl 70% EtOH to wash

20. Spin for 5 min at top speed

21. Remove supernatant and add 500 µl RNA resuspension buffer

22. Incubate at 65 °C to dissolve pellet
Pipetting up and down can help as well.

23. Add 200 µl 2-Butoxyethanol an mix well
In this first step we precipitate polysaccharides at a low 2-BE concentration.

24. Incubate on ice for at least 3 hours.
The publication incubates for more than 30 min, it seems both is working.

25. Spin for 10 min at max. speed at 4 °C
It will form a brown pellet containing the sugars.

26. Transfer supernatant to a fresh eppi

27. Add 300 µl 2-Butoxyethanol and mix well
In this second step we precipitate nucleotides at a high 2-BE concentration.

28. Incubate on ice over night.
The publication incubates for more than 30 min and I extended it up to 24 hours, it seems as if both is working.

29. Spin for 10 min at max. speed at 4 °C
It will form a brown pellet containing the nucleotides.

30. Discard the supernatant and add 500 µl 70% EtOH

31. Spin for 5 min at max. speed at 4 °C
The pellet does not look different.

32. Discard the supernatant and let pellet briefly dry
Not too long, over dried nucleotides are difficult to dissolve in water.

33. Dissolve in 1 ml water (and transfer to an eppi)

34. Precipitate RNA by adding 335 µl 8 M LiCl

35. Incubate at 4 °C over night
Lithiumchlorid precipitates RNA, the timing seems to be critical as well. The majority should be precipitated after four hours.

36. Spin for 30 min at max. speed at 4 °C
It will form a brown pellet containing the nucleotides.

37. Discard the supernatant and add 100 µl 70% EtOH

38. Spin for 5 min at max. speed
It will form a brown pellet containing the nucleotides.

39. Discard the supernatant and dissolve in 200 µl water

40. Add 20 µl 3 M NaAcetate (pH 5.2) and 500 µl 96% ethanol

41. Spin for 5 min at max. speed
It will form a brown pellet containing the nucleotides.

42. Store at -80 °C until frozen

43. Spin for 30 min at top speed at 4 °C

44. Remove supernatant and add 100 µl 70% EtOH to wash

45. Spin for 5 min at top speed

46. Remove supernatant and redissolve 50 µl water

47. Incubate at 65 °C to dissolve pellet
Pipetting up and down can help as well.

Known Issues:

- This extraction method takes three days and lots of hand on time.
- The buffer is a mess and some compounds are difficult to get.

References and Comments:

This protocol should allow to extract RNA from difficult tissues, which are rich on sugars and phenolic compounds. I got the protocol from J. Schaart and tried it out for strawberry roots. I got great RNA, but still the qPCR had some problems. If you have problems extracting RNA, give it a try, but it is a very labor intensive protocol.
The magic step is the precipitation of sugars with 2-BE. Alternatively you could use your own extraction buffer and just add the 2-BE steps to your protocol. Good luck!

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RNA Isolation from Recalcitrant Plant Tissue.
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