

RNA Midiprep 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. 4 g tissue into a fine powder
3. Transfer grinded powder into a 50 ml tube
4. Add **30 ml of extraction buffer** and vortex well
Careful, pressure may build up!
5. Spin for **10 min** at 3000 rpm
6. Transfer top phase to a fresh 50 ml tube
7. Add **15 ml chloroform** and vortex well
8. Spin for **5 min** at 3000 rpm
9. Transfer top phase to a fresh 50 ml tube
10. Add **15 ml phenol / chloroform** and vortex well
11. Spin for **10 min** at 3000 rpm
12. Transfer top phase to a fresh 50 ml tube
13. Add **15 ml chloroform** and vortex well
14. Spin for **5 min** at 3000 rpm
15. Transfer top phase to a fresh 50 ml tube
16. Add **1.2 ml 3 M NaAc and 30 ml 96% EtOH** and vortex well
17. Store at -80 °C until frozen
18. Spin for **5 min** at 4500 rpm at **4 °C**
19. Remove supernatant and add **15 ml 70% EtOH** to wash
20. Spin for **5 min** at 4500 rpm
21. Remove supernatant and add **15 ml RNA resuspension buffer**
22. Incubate at 65 °C to dissolve pellet
23. Add **6 ml 2-Butoxyethanol** and mix well
24. Incubate on ice for at least 3 hours.
25. Spin for **10 min** at 4500 rpm at **4 °C**
26. Transfer supernatant to a fresh 50 ml tube
27. Add **9 ml 2-Butoxyethanol** and mix well
28. Incubate on ice over night.

29. Spin for **10 min** at 4500 rpm at **4 °C**
30. Discard the supernatant and add **5 ml 70% EtOH**
31. Spin for **5 min** at 4500 rpm 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:

Triisopropyl naphthalene 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%
Polyvinylpyrrolidone (PVP) (M.W. 40,000)	5 g	1%
Polyvinylpolypyrrolidone (PVPP)	15 g	3%
add water to 500 ml		

Autoclave for 15 min, then add:

chloroform	500 ml	
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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 where the tip was cut off. Always add beta-mercaptoethanol fresh.

Before use add Mercaptoethanol:

for 50 ml Buffer add 250 μ l Mercaptoethanol

for 30 ml Buffer add 150 μ l Mercaptoethanol

for 10 ml Buffer add 50 μ l Mercaptoethanol

for 5 ml Buffer add 25 μ l Mercaptoethanol

for 1 ml Buffer add 5 μ l 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. 4 g 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 50 ml tube

4. Add 30 ml of extraction buffer and vortex well

Careful, pressure may build up!

5. Spin for 10 min at 3000 rpm

The chloroform is already in the extraction buffer.

6. Transfer top phase to a fresh 50 ml tube

7. Add 15 ml chloroform and vortex well

8. Spin for 5 min at 3000 rpm

9. Transfer top phase to a fresh 50 ml tube

10. Add 15 ml 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 3000 rpm

12. Transfer top phase to a fresh 50 ml tube

13. Add 15 ml chloroform and vortex well

14. Spin for 5 min at 3000 rpm

15. Transfer top phase to a fresh 50 ml tube

16. Add 1.2 ml 3 M NaAc and 30 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 4500 rpm at 4 °C

19. Remove supernatant and add 15 ml 70% EtOH to wash

20. Spin for 5 min at 4500 rpm

21. Remove supernatant and add 15 ml RNA resuspension buffer

22. Incubate at 65 °C to dissolve pellet

Pipetting up and down can help as well.

23. Add 6 ml 2-Butoxyethanol and 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 4500 rpm at 4 °C

It will form a brown pellet containing the sugars.

26. Transfer supernatant to a fresh 50 ml tube

27. Add 9 ml 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 4500 rpm at 4 °C

It will form a brown pellet containing the nucleotides.

30. Discard the supernatant and add 5 ml 70% EtOH

31. Spin for 5 min at 4500 rpm 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!

How to cite this page in publications:

This document can be cited like this:

Untergasser A. "RNA Midiprep using 2-Butoxyethanol" *Untergasser's Lab*. Summer 2008. (include here the date when you accessed these page).

<http://www.untergasser.de/lab/protocols/midiprep_rna_butoxyethanol_long_v1_0.htm>.

It is heavily based on the following publication. Maybe you prefer to cite the original paper: Schultz DJ, Craig R, Cox-Foster DL, Mumma RO, Medford JI.

RNA Isolation from Recalcitrant Plant Tissue.

Plant Molecular Biology Reporter, 310-316, 12 (4) 1994.

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