RNA Miniprep using hot phenol

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ATTENTION: This is a low priced protocol. Be careful - handling hot phenol is dangerous.

1. **Add 1:1 phenol (pH 6.6)** to the RNA extraction buffer
2. Heat the extraction mix to **90 °C**
3. Grind tissue into a fine powder
4. Transfer tissue-powder to new eppi
5. Add **1.0 ml hot extraction mix** and vortex very well
6. Add **0.5 ml chloroform** and mix very well
7. Spin down at max speed for **15 min**
8. Transfer **upper phase** into a new eppi
9. Add **1:1 chloroform** and mix well
10. Spin down at max speed for **2 min**
11. Transfer **upper phase** into a new eppi
12. Add **1/3 vol 8 M LiCl** and mix well
13. Precipitate the RNA for at least **4 h at 4 °C**
14. Spin down at max speed for **30 min at 4 °C**
15. Keep the **pellet**, discard supernatant
16. Add **750 µl 70% ethanol** and mix well
17. Spin down at max speed for **2 min**
18. Keep the **pellet**, discard supernatant
    If no DNase treatment is required, go directly to step 32.
19. Dissolve pellet in **90 µl water** for 15 min at 65 °C
20. Add **10 µl DNase buffer** and **1 µl DNase**
21. Incubate at **37 °C for 30 min**
22. Add **150 µl chloroform** and mix well
23. Spin down at max speed for **2 min**
24. Transfer **upper phase** into a new eppi
25. Add **10 µl 3 M sodium acetate pH 5.2** and **250 µl 100% ethanol**
26. Incubate **over night** at **-20 °C**
27. Spin down at max speed for **20 min** to pellet RNA
28. Keep the **pellet**, discard supernatant
29. Add **750 µl 70% ethanol** and mix well
30. Spin down at max speed for **2 min**

31. Keep the **pellet**, discard supernatant
32. Dissolve pellet in **30 µl water** for 15 min at 65 °C
33. Store RNA on ice at the bench or at -80 °C for long term storage

Buffers and Solutions

RNA extraction buffer

		100 ml	500 ml
LiCl	100 mM	4.2 g	21.0 g
3 M NaAc (sodium acetate), pH 5.2	100 mM	3.3 ml	16.7 ml
0.5 M EDTA, pH 8.0	10 mM	2 ml	10 ml
SDS	1 %	1 g	5 g
water		add to 100 ml	add to 500 ml

Before use add 1:1 phenol (pH 6.6)

Commented Protocol:

1. Add 1:1 phenol (pH 6.6) to the RNA extraction buffer

The buffer is not stable, use it within one day.

2. Heat the extraction mix to 90 °C

Careful, hot phenol is nasty.

3. Grind 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.

4. Transfer tissue-powder to new eppi

It works best if the eppi is precooled.

5. Add 1.0 ml hot extraction mix and vortex very well

6. Add 0.5 ml chloroform and mix very well

After this step I wait till I processed all samples.

7. Spin down at max speed for 15 min
Handle the tubes carefully to not disturb the interphase.

8. Transfer upper phase into a new eppi
It is no problem if you take a little chloroform. But do not take anything of the interphase.

9. Add 1:1 chloroform and mix well

10. Spin down at max speed for 2 min
This time no white interface should form.

11. Transfer upper phase into a new eppi
Try to take none of the chloroform at this step.

12. Add 1/3 vol 8 M LiCl and mix well
This will precipitate the RNA only.

13. Precipitate the RNA for at least 4 h at 4 °C
It probably helps to reduce the temperature. But do not extend it over 4 hours, then unwanted contaminants may also precipitate.

14. Spin down at max speed for 30 min at 4 °C

15. Keep the pellet, discard supernatant
The pure RNA pellet might be transparent and hardly visible.

16. Add 750 µl 70% ethanol and mix well
To wash off the salts.

17. Spin down at max speed for 2 min

18. Keep the pellet, discard supernatant
If no DNase treatment is required, go directly to step 32.

19. Dissolve pellet in 90 µl water for 15 min at 65 °C
Most of the time pipetting up and down is sufficient.

20. Add 10 µl DNase buffer and 1 µl DNase
To get rid of contaminating DNA.

21. Incubate at 37 °C for 30 min

22. Add 150 µl chloroform and mix well

23. Spin down at max speed for 2 min
This time no white interface should form.

24. Transfer upper phase into a new eppi
Try to take none of the chloroform at this step.

25. Add 10 µl 3 M sodium acetate pH 5.2 and 250 µl 100% ethanol

26. Incubate over night at -20 °C

27. Spin down at max speed for 20 min to pellet RNA
Just to be extra clean on this step. If you are in time pressure and never get a pellet you may skip this step and the next at own risk.

28. Keep the pellet, discard supernatant
The pure RNA pellet might be transparent and hardly visible.

29. Add 750 µl 70% ethanol and mix well
To wash off the salts.

30. Spin down at max speed for 2 min

31. Keep the pellet, discard supernatant

32. Dissolve pellet in 30 µl water for 15 min at 65 °C
Most of the time pipetting up and down is sufficient.

33. Store RNA on ice at the bench or at -80 °C for long term storage

References and Comments:

This is a basic protocol used in the plant field to extract RNA from all kinds of tissues. It is popular because it works really well.

How to cite this page in publications:

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<http://www.untergasser.de/lab/protocols/miniprep_rna_hot_phenol_v1_0.htm>.