RNA Miniprep using CTAB

by A. Untergasser (contact address and download at www.untergasser.de/lab) - Version: 1.0

ATTENTION: This is a low priced protocol. Use it preferably! It is a great protocol to extract RNA from plants.

1. **Add 1% beta-mercaptoethanol** to the CTAB-buffer
2. Prepare **600 µl CTAB-buffer** in an eppi
3. Grind tissue into a fine powder
4. Transfer tissue-powder to the eppi and mix shortly
5. Add **600 µl chloroform** and mix very well
6. Spin down at max speed for **2 min**
7. Transfer **upper phase** into a new eppi
8. Add **600 µl chloroform** and mix well
9. Spin down at max speed for **2 min**
10. Transfer **upper phase** into a new eppi
11. Add **equal amounts of isopropanol (1:1)** and mix well
12. Spin down at max speed for **15 min**
13. Keep the **pellet**, discard supernatant
14. Add **600 µl 70% ethanol** to wash and mix well
15. Spin down at max speed for **5 min**
16. Keep the **pellet**, discard supernatant
17. Dissolve pellet in **90 µl water** for 15 min at 65 °C
    If you like to use columns to purify the RNA: Dissolve the pellet in a suitable amount of water to match your kit and continue with the kit from here on.
18. Spin down at max speed for **5 min** to remove debris
19. Transfer the **liquid** into a new eppi
20. Add **30 µl 8 M LiCl** and mix well
21. Precipitate the RNA for at least **20 min at -20 °C**
22. Spin down at max speed for **30 min at 4 °C**
23. Keep the **pellet**, discard supernatant
24. Add **100 µl 70% ethanol** and mix well
25. Spin down at max speed for **2 min**
26. Keep the **pellet**, discard supernatant
27. Dissolve pellet in **20 µl water** for 15 min at 65 °C
28. Spin down at max speed for **5 min** to remove debris
29. Transfer the **liquid** into a new eppi
30. Store RNA on ice at the bench or at -80 °C for long term storage

Buffers and Solutions

CTAB Buffer

		100 ml	500 ml
CTAB (Cetyl Trimethyl Ammonium Bromide)	2%	2 g	10 g
NaCl	1.4 M	8.1 g	40.6 g
0.5 M EDTA, pH 8.0	20 mM	4 ml	20 ml
1 M TRIS, pH 8.0	100 mM	10 ml	50 ml
Polyvinylpyrolidone (PVP40) (M.W. 40,000)	2%	2 g	10 g
water		add to 100 ml	add to 500 ml

The buffer takes some time to dissolve. Some people like to autoclave it before use (me), others prefer to make it fresh.

Before use add 1 % beta-mercaptoethanol!

Commented Protocol:

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

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

2. Prepare 600 µl CTAB-buffer in an eppi

Directly go to the next step after each sample.

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 the eppi and mix shortly

Do not take too much or too few. I take ca. 100 mg and obtain 10 µg RNA in the end. If you take too much, the lysis will not work, if you take to few, the RNA will not precipitate efficient enough.

5. Add 600 µl chloroform and mix very well

After this step I wait till I processed all samples.

6. Spin down at max speed for 2 min

Handle the eppis carefully to not disturb the interphase.

7. 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.

8. Add 600 µl chloroform and mix well

9. Spin down at max speed for 2 min

This time no white interface should form.

10. Transfer upper phase into a new eppi

Try to take none of the chloroform at this step.

11. Add equal amounts of isopropanol (1:1) and mix well

This precipitates all nucleic acids.

12. Spin down at max speed for 15 min

13. Keep the pellet, discard supernatant

The pellet should be white and easily visible.

14. Add 600 µl 70% ethanol to wash and mix well

This washes off the salts.

15. Spin down at max speed for 5 min

Probably even 1 min would be sufficient.

16. Keep the pellet, discard supernatant

Do not dry the pellet at this step.

17. Dissolve pellet in 90 µl water for 15 min at 65 °C

If you like to use columns to purify the RNA: Dissolve the pellet in a suitable amount of water to match your kit and continue with the kit from here on.
If I continue with the RNeasy kit from Qiagen I dissolve the pellet in 75 µl water, add 340 µl RTL and 200 µl 100% EtOH and then follow the kit instructions.

18. Spin down at max speed for 5 min to remove debris

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.

19. Transfer the liquid into a new eppi

20. Add 30 µl 8 M LiCl and mix well

This will precipitate the RNA only.

21. Precipitate the RNA for at least 20 min at -20 °C
It probably helps to extend this step. But do not extend it over 4 hours, then unwanted contaminants may also precipitate.

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

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

24. Add 100 µl 70% ethanol and mix well
To wash of the salts.

25. Spin down at max speed for 2 min

26. Keep the pellet, discard supernatant

27. Dissolve pellet in 20 µl water for 15 min at 65 °C
Most of the time pipetting up and down is sufficient.

28. Spin down at max speed for 5 min to remove debris
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.

29. Transfer the liquid into a new eppi

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

Known Issues:

- Works well on Tobacco, Tomato and Arabidopsis.
- If you don't get RNA with this protocol, search for protocols specific for your plant and be prepared for difficulties. You can try the 2-Butoxyethanol protocol.

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. I did it several times and combined it with column purification, the RNA was always great.

How to cite this page in publications:

This document can be cited like this:

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<http://www.untergasser.de/lab/protocols/miniprep_rna_ctab_v1_0.htm>.

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