RNAprep - Trizol combined with Columns

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

This protocol is quite good because it allows to make high amounts of RNA which are very clean due to the column step. It works with all tissues, rat liver, bacteria or plant tissue.

1. Homogenize you tissue
2. Add 500 µl Trizol to the tissue
   Do not use too much material. This amount of trizol can handle up to 50 mg tissue, 5 cm^2 tissue culture area or 5 x 10^6 animal/plant/yeast/bacteria cells.
   The sample volume should not exceed 50 µl.
3. Optional: spin at 12000 x g for 10 min at 4 °C
4. Optional: transfer the supernatant to a new tube
5. Incubate for 5 min at room temperature
6. Add 100 µl of chloroform and mix well
7. Incubate for 2 min at room temperature
8. Spin down at 12000 x g for 15 min at 4 °C
9. Transfer the transparent upper phase to a new tube
10. Add equal volume (300 µl) of 70 % ethanol and mix well
11. Transfer to a RNeasy Mini spin column
12. Spin down 15 sec at 8000 x g and discard flow-through
13. Add 350 µl Buffer RW1 to the column
    If you don't need a DNAse treatment use 700 µl Buffer RW1 and go directly to step 20.
14. Spin down 15 sec at 8000 x g and discard flow-through
15. Mix 10 µl Qiagen DNase I with 70 µl Buffer RDD
    Mix very carefully because DNase I is especially sensitive to physical denaturation.
16. Add the mix to the column
17. Incubate for 30 min at room temperature
18. Add 350 µl Buffer RW1 to the column
19. Incubate for 5 min at room temperature
20. Spin down 15 sec at 8000 x g and discard flow-through
21. Add 500 µl Buffer RPE to the column
22. Spin down 15 sec at 8000 x g and discard flow-through
23. Add 500 µl Buffer RPE to the column
24. Spin down 1 min at 8000 x g and discard flow-through
25. Place column in a fresh collection tube
26. Spin down 2 min at max speed to dry the column

27. Place column in a 1.5 ml eppi
28. Pipett 30 µl RNase-free water directly on the membrane
29. Incubate at room temperature for 1 min
30. Spin down 1 min at max speed to elute the RNA

Materials needed:

Trizol (# 15596-018) by Invitrogen
RNeasy Mini Kit (# 74104) by Qiagen
DNase I Kit (# 79254) by Qiagen

Commented Protocol:

1. Homogenize you tissue

Check out the "How to homogenize tissues" for details. For animal cells you can use a mixer, plant tissues are usually frozen in liquid nitrogen and grinded in a mortar. Bacteria and tissue culture cells don't need any homogenisation.

2. Add 500 µl Trizol to the tissue

Do not use too much material. This amount of trizol can handle up to 50 mg tissue, 5 cm^2 tissue culture area or 5 x 10^6 animal/plant/yeast/bacteria cells.
The sample volume should not exceed 50 µl.

3. Optional: spin at 12000 x g for 10 min at 4 °C

If the material contains lots of fat, proteins, polysaccharides or extracellular material like fat tissue, muscle or certain plant tissues, this step can reduce the debris. I usually skip this step, but if you have problems with the isolation you may try it out.

4. Optional: transfer the supernatant to a new tube

5. Incubate for 5 min at room temperature

6. Add 100 µl of chloroform and mix well

7. Incubate for 2 min at room temperature

8. Spin down at 12000 x g for 15 min at 4 °C

Now it will seperate into a lower red phenol phase, a interphase and a transparent water phase with the RNA (on TOP!).

9. Transfer the transparent upper phase to a new tube

10. Add equal volume (300 µl) of 70 % ethanol and mix well

11. Transfer to a RNeasy Mini spin column
Following the addition of ethanol a precipitate may form. This should be resuspended and also transfered to the column. If the sample is mor than 700 µl, load it in several steps.

12. Spin down 15 sec at 8000 x g and discard flow-through

13. Add 350 µl Buffer RW1 to the column
If you don't need a DNAse treatment use 700 µl Buffer RW1 and go directly to step 20.

14. Spin down 15 sec at 8000 x g and discard flow-through

15. Mix 10 µl Qiagen DNase I with 70 µl Buffer RDD
Mix very carefully because DNase I is especially sensitive to physical denaturation.

16. Add the mix to the column

17. Incubate for 30 min at room temperature

18. Add 350 µl Buffer RW1 to the column

19. Incubate for 5 min at room temperature

20. Spin down 15 sec at 8000 x g and discard flow-through

21. Add 500 µl Buffer RPE to the column

22. Spin down 15 sec at 8000 x g and discard flow-through

23. Add 500 µl Buffer RPE to the column

24. Spin down 1 min at 8000 x g and discard flow-through

25. Place column in a fresh collection tube

26. Spin down 2 min at max speed to dry the column

27. Place column in a 1.5 ml eppi

28. Pipett 30 µl RNase-free water directly on the membrane

29. Incubate at room temperature for 1 min

30. Spin down 1 min at max speed to elute the RNA

Measure the RNA concentration. Don't forget that RNA uses the factor 40 and not 50 (DNA) for concentration calculation. A_{260}/A_{280} should be between 1.8 - 2.1.

Known Issues:

- Be careful when working with RNA and stick to the guidelines in "How to work with RNA" to avoid contamination with RNAse. Always wear gloves!

References and Comments:

This is a protocol I created because I did it once in my time in Heidelberg like that. I should also mention that Qiagen has a kit in stock (RNeasy Lipid Tissue Mini Kit) which does exactly the same using "QIAzol Lysis Reagent". I wrote the protocol because most labs have Trizol and the "RNAeasy Mini kit" in house so you are ready to go.
I did it several times and was very happy with the results.

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