RNA Extraction Protocol Using Zymo kit Direct-zol RNA MiniPrep Plus

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Catalog #: 50 preps - R2070, 200 preps - R2072; TriReagent 200ml - R2050-1-200
Adapted to extract 142mm filters.
Perform the DNase I treatment in column as described with kit protocol.

Buffer preparation – when using the kit for the first time.

*Use 100% ethanol for both buffers.

1. Add 10ml or 40ml ethanol (95-100%) to the 40ml or 160ml Direct-zol RNA PreWash concentrate, respectively.

2. Add 48ml 100% ethanol (52ml 95% ethanol) to the 12ml RNA Wash Buffer concentrate or 192ml 100% ethanol (208ml 95% ethanol) to the 48ml RNA Wash Buffer concentrate.

Sample Preparation

1. Clean fume hood and pipettes with 70% ethanol and RNase Zap.

2. Clean forceps and scissors with ethanol and flame.

3. Prepare bead mix to add to sample.
    - Approx. 125µl of small (100µm) and medium zirconia beads (500µm)
    - Approx. 250µl large glass beads (425-600µm)

4. Add bead mix to 50mL tube.

5. Add 3ml TRI Reagent to tube.

6. Cut 1 filter using sterile forceps and scissors on combusted foil over ice.

7. Place filter pieces in 50ml tube.

8. Parafilm tube.

9. Bead beat for 2min.

10. Spin 50ml tube for 1 minute at 4,000 rpm. Decant liquid to new 15ml tube.

RNA Purification

11. Add an equal volume 100% ethanol. Mix thoroughly.
    - Vortex for 40 seconds.
    - Do a quick spin after to get Tri-Reagent off the cap.

12. Transfer the mixture into a Zymo-Spin IIC Column attached to vacuum manifold, OR if placing column in collection tube and using centrifuge:
    - load 700ul into column. Centrifuge for 30sec at 16,000 x g. Discard flow-through.
    - To process all the liquid, load 700µl into column and repeat step 8.
    - **LAST SPIN** do for 1min.
    - If using vacuum manifold, spin all columns for 1min once all liquid has been passed through to ensure complete removal of Tri Reagent and ethanol.

13. Transfer the column into a new collection tube.

DNase I treatment

14. Add 400µl RNA Wash Buffer to the column and centrifuge for 30sec at 16,000 x g. Discard flow-through.

15. In an RNase-free tube, add 5µl DNase I (6 U/µl), 75µl DNA Digestion Buffer and mix by gentle inversion. Add the mix directly to the column matrix.
    - For 6 samples combine 450ul DNA Digestion Buffer and 30ul DNase 1.
    - Mix by flicking the tube since inverting didn't work well.
    - Quickly spin down tube.

16. Add the DNase mix directly to the column matrix.
    - Add 80ul DNA Digestion Buffer/DNase 1 mix to each sample.

17. Incubate at room temperature (20-30˚C) for 15min.

RNA Purification continued

18. Add 400ul Direct-zol RNA PreWash to the column and centrifuge for 30sec at 16,000 x g.

19. Discard the flow-through.

20. Repeat steps 17 and 18.

21. Add 700µl RNA Wash Buffer to the column and centrifuge for 30sec at 16,000x g.

22. Discard the flow-through and centrifuge again for 1.5min at 16,000 x g to ensure complete removal of the wash buffer.

23. Transfer the column carefully into an RNase-free tube.

24. To elute RNA, add 50µl of DNase/RNase-Free Water directly to the column matrix and centrifuge for 30sec at 16,000 x g.

25. Repeat step 23 again for a total of 100 µl.

When purification is complete, quant sample with Nanodrop and store in -80C freezer.