

Total RNA Cleanup with Dnase Digestion using Qiagen RNeasy Protocol

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A. Required reagents:

- ≤100 ug RNA isolated by any method other than Qiagen RNeasy method
- DEPC-treated water (Ambion)
- RNeasy RNA Isolation Kit (Qiagen)
- RNase-Free Dnase Set (Qiagen)
- β-Mercaptoethanol (β-ME) (Sigma)
- Absolute ethanol (Ultrapure) (American Bioanalytical)

B. Equipment and supplies:

- Microcentrifuge with 1.5 ml tube rotor
- Vortex mixer
- Micropipettors
- Aerosol-barrier tips
- Microcentrifuge tubes
- Vortex mixer
- Powder-free gloves

I. TOTAL RNA CLEAN-UP

- * Maximum binding capacity of RNeasy mini spin column is 100 µg of RNA.
- * Buffer RLT may form precipitate upon storage. If necessary, warm to redissolve.
- * Add 10 µl β-ME per 1 ml of buffer RLT just before use.
- * Buffer RPE is supplied as a concentrate. Before using the first time, add 4 volumes of absolute ethanol as indicated on the bottle to obtain working solution.
- * Prepare DNase I stock solution according to package insert.

1. Adjust sample volume to 100 µl with DEPC-treated water. Add 350 µl Buffer RLT to the sample, and mix thoroughly.
2. Add 250 µl absolute ethanol to the lysate. Mix well by pipetting 2 to 3 times.
3. Apply 700 µl of sample, including any precipitate, to an RNeasy mini spin column sitting in a 2 ml collection tube. Incubate column for 5 min. Centrifuge for 15 sec at full speed. If

the volume of the mixture exceeds 700 μ l, successively load aliquots onto the RNeasy column and centrifuge as above. Reuse the collection tube in Step 4.

4. Reload the sample on the RNeasy column a second time to increase binding of RNA to the RNeasy membrane. Centrifuge for 15 sec at full speed.

5. Transfer RNeasy column into a new 2 ml collection tube. Save flow through until sample quantitation is completed.

6. Pipet 350 μ l Buffer RW1 onto column. Incubate 1 min. Spin for 15 sec at maximum speed. Discard flow-through.

7. Add 10 μ l Dnase I stock solution to 70 μ l Buffer RDD. Mix by gently inverting the tube. Do not vortex.

8. Pipet 80 μ l Dnase I incubation mix directly onto column. Incubate at room temperature for 15 min.

9. Pipet 350 μ l Buffer RW1 onto column. Spin at maximum speed for 15 sec. Discard wash.

10. Repeat wash of 350 μ l Buffer RW1. Incubate for 3-5 min. Spin at maximum speed for 15 sec. Discard wash.

11. Wash column with 350 μ l Buffer RW1 for a final time. Spin at maximum speed for 15 sec.

12. Pipette 500 μ l Buffer RPE onto RNeasy column and centrifuge for 15 sec at maximum speed to wash. Discard flow through and reuse the collection tube in Step 13.

13. Pipette 500 μ l RPE buffer onto RNeasy column. Centrifuge for 2 min at maximum speed to dry the RNeasy membrane.

14. Place the RNeasy spin column in a new 2 ml collection tube. Discard collection tube and filtrate. Centrifuge at full speed for 2 min.

*It is important to dry the RNeasy membrane since residual ethanol may reduce the recovery of RNA and also may interfere with subsequent reactions.

15. Transfer RNeasy column into a new 1.5 ml collection tube. Let sit with lid open for 10 min to completely evaporate ethanol.

16. Add 40 μ l of DEPC-treated water directly onto the center of the RNeasy membrane. Wait 2 min. Centrifuge for 1 min at maximum speed to elute.

17. In order to recover all of the bound RNA, repeat step 16 with 30 μ l of DEPC-treated water.

18. Use spectrophotometric analysis to measure RNA yield. Dilute 1 μ l of RNA with 39 μ l of DEPC-treated water (1:40 dilution). Using a 10 μ l microcuvette, take OD at 260 nm and 280 nm to determine sample concentration and purity. The A_{260}/A_{280} ratio should be above 1.8. Apply the convention that 1 OD at 260 equals 40 μ g/ml RNA.

19. Run 0.5-1 μ g of RNA on native 1 % agarose gel or 0.1-0.5 μ g on an Agilent Bioanalyzer chip to assess the quality of RNA.

20. Important: It is extremely important to start microarray experiments with very good quality of RNA. The A_{260}/A_{280} ratio should be above 1.8. The gel electrophoresis pattern should reveal two major bands of 28 S and 18 S RNA and no smear from genomic DNA (See Figure 1 below).

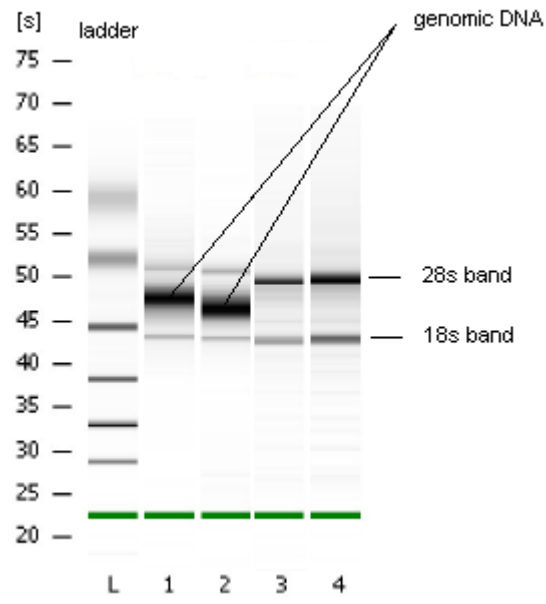


Figure 1: Bioanalyzer gel-image pattern of total RNA