Total RNA Cleanup with Dnase Digestion using Qiagen RNeasy Protocol

Please do not make copies of or distribute this protocol.

A. Required reagents:
   - ≤100 µg 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 (Utrapure) (American Bioanalytical)

B. Equipment and supplies:
   - Microcentrifuge with 1.5 ml tube rotor
   - Vortex mixer
   - Micropipette tips
   - 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 μl, 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.


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

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


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

12. Pipette 500 μl Buffer RPE onto RNeasy column and incubate for 5 minutes. Centrifuge for 15 sec at maximum speed to wash. Repeat Step 12 one more time. Discard flow through and reuse the collection tube in Step 13.

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


15. Open the column cap and spin at full speed (maximum speed) for 5 minutes with the caps open.

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

16. Transfer RNeasy column into a new 1.5 ml collection tube. Let sit with lid open for 5 to 10 min to completely evaporate ethanol.

17. Add 40 μl of DEPC-treated water directly onto the center of the RNeasy membrane. Wait 5 min. Centrifuge for 1 min at maximum speed to elute.

18. Elute a second time by pipetting the flow-through in the collection tube (~36 ul) back onto the Spin Column membrane. Place the spin column back into the collection tube and incubate at room temperature for two minutes. Spin at maximum speed for 1 minute.

19. Use spectrophotometric analysis to measure RNA yield. Take OD at 230 nm, 260 nm and 280 nm to determine sample concentration and purity. The A$_{260}$/A$_{280}$ ratio should be above 1.8. The A$_{260}$/A$_{230}$ should be above 1.8. Apply the convention that 1 OD at 260 equals 40 μg/ml 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](Image)