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RNeasy Total RNA Cleanup Protocol

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A. Required reagents:
   - RNA isolated by any method other than Qiagen RNeasy method
   - DEPC-treated water (Ambion)
   - RNeasy RNA Isolation Kit (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.

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. Pipette 500 μl 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 6.

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

7. 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.

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

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

10. In order to recover all of the bound RNA, repeat step 9 with 30 μl of DEPC-treated water.

11. Use spectrophotometric analysis to measure RNA yield. Dilute 1 μl of RNA with 39 μl of DEPC-treated water (1:40 dilution). Using a 10 μl 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 μg/ml RNA.

12. Run 0.5 to 1 μg of RNA on native 1 % agarose gel or 0.1 to 0.5 μg on an Agilent Bioanalyzer chip to assess the quality of RNA.

**13. 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-url)