

## RNeasy Total RNA Cleanup Protocol

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Please do not make copies of or distribute this protocol.

A. Required reagents:

RNA isolated by any method other than Qiagen RNeasy method

DEPC-treated water (Ambion)

RNeasy RNA Isolation Kit (Qiagen)

$\beta$ -Mercaptoethanol ( $\beta$ -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  $\mu$ g of RNA.

\* Buffer RLT may form precipitate upon storage. If necessary, warm to redissolve.

\* Add 10  $\mu$ l  $\beta$ -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  $\mu$ l with DEPC-treated water. Add 350  $\mu$ l Buffer RLT to the sample, and mix thoroughly.

2. Add 250  $\mu$ l absolute ethanol to the lysate. Mix well by pipetting 2 to 3 times.

3. Apply 700  $\mu$ l 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  $\mu$ 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. Pipette 500  $\mu$ 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 6.

6. Pipette 500  $\mu$ l 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  $\mu$ 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.

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

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

12. Run 0.5 to 1  $\mu$ g of RNA on native 1 % agarose gel or 0.1 to 0.5  $\mu$ g 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).**

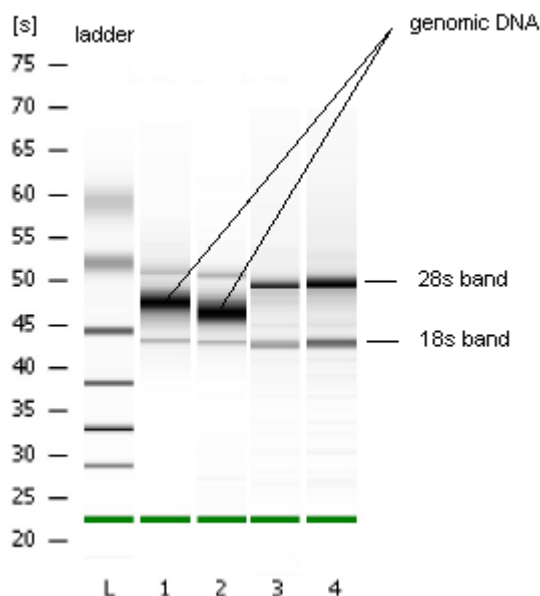


Figure 1: Bioanalyzer gel-image pattern of total RNA