



## RNA Extraction from Mammalian Tissues

### Reagents

**Choose the most appropriate kit for your sample.** Consult the Qiagen web site for more specifics, or call Qiagen technical support (1 (800) 362-7737):

- **Qiagen RNeasy® Mini Kit (12):** Qiagen (Cat. No. 74104). Yields <100 µg RNA from 0.5 - 30 mg tissue or  $1 \times 10^5$  to  $1 \times 10^7$  cells.
- **Qiagen RNeasy® Midi Kit (12):** Qiagen (Cat. No. 75142). Yields <1 mg RNA from 20 - 250 mg tissue or  $5 \times 10^6$  -  $1 \times 10^8$  cells.
- **Qiagen RNeasy® Maxi Kit (12):** Qiagen (Cat. No. 75162). Yields <6 mg RNA from 150 mg – 1 g of tissue or  $5 \times 10^7$  –  $5 \times 10^8$  cells.

**TRIzol® Reagent** (a ready to use mixture of phenol, guanidine isothiocyanate, red dye and other proprietary components): Invitrogen (Cat. No. 15596-026)

**TRIzol® LS Reagent** (recommended for liquid samples e.g. FACS sorted cells): Invitrogen (Cat. No. 10296-010)

**Chloroform:** Sigma (Cat. No. C-2432)

**RNaseZap® RNase Decontamination Solution, 250 ml:** Ambion (Cat. No. 9780)

**RNase-free Water:** Ambion (Cat. No. 9932 or 9922)

**100 % (200 proof) Ethanol:** Pharmco (Cat. No. 111ACS200)

**70% Ethanol** (in RNase-free H<sub>2</sub>O)

**50 ml Falcon Tubes** (BD Cat. No. 352070). 9400 RCF rating. Required for Maxi Kit.

**15 ml Falcon Tubes** (BD Cat. No. 352097). 6000 RCF rating. Required for Midi Kit.

**1.7 ml Microcentrifuge Tubes** (Denville Scientific, Cat. No. C-2170). Required for Mini Kit.

**Agilent 2100 bioanalyzer:** (Agilent Technologies, Cat. No. G2940CA)

**Agilent RNA 6000 Nano Kit:** (Agilent Technologies, Cat. No. 5067-1511). For RNA in the concentration range of 25–500 ng µl<sup>-1</sup>

**Agilent RNA 6000 Pico Kit:** (Agilent Technologies, Cat. No. 5067-1513). For RNA in the concentration range of 50–5000 pg µl<sup>-1</sup>

**NOTE:** Where possible avoid use of carrier molecules in preparing your RNA. If working with extremely small samples then we recommend Poly(dI-dC) (Sigma, Cat. No. P4929). Never use glycogen or tRNA.

**NOTE:** We do not recommend the use of the RNAlater RNA stabilization reagent (this should not be confused with Buffer RLT). Direct disruption of the tissue or cells in Buffer RLT or Trizol yields the best results in our experience. If the tissue has been stored in RNAlater, the tissue must first be removed and placed into Buffer RLT and immediately disrupted.

**NOTE:** We do not recommend DNase treatment of RNA samples for microarray analysis.

**NOTE:** If you are not experienced with RNA isolation, please read the literature and tips found on Ambion's website: <http://www.ambion.com/techlib/basics/rnaisol/index.html>.

**NOTE:** Alternatively, Invitrogen has released the “**TRIzol® Plus RNA Purification System**” (Cat. No. 12183-555), in which they provide both TRIzol and their own purification columns, similar to this method.

## RNA Extraction Protocol for Standard Samples

### Sample preparation and homogenization

Gloves should be worn at all times and follow standard RNA handling techniques. Lysis of the sample in **TRIzol**<sup>®</sup> works well and may give higher yields due to better lysis than can be achieved with the Buffer RLT provided in the Qiagen RNeasy kits.

- a. **Cells.** To a pellet of cells ( $<1 \times 10^7$  cells) add 1 ml **TRIzol** or for direct lysis of cells grown in a monolayer, add 1 ml **TRIzol** ( $<10$  cm diameter dish) and collect cell lysate with a rubber policeman, transferring to a 2.0 ml microcentrifuge tube.
- b. **Sorted Cells.** The issue with extracting RNA from sorted cells is the volume of cells after sorting must not dilute the denaturing agent beyond its ability to denature RNase. As such we recommend the use of **TRIzol LS** which remains active when diluted up to 1:3. If possible, sort cells directly into 750  $\mu$ l of TRIzol LS (sample volume must not exceed 250  $\mu$ l – if this is a problem use more reagent and adjust the protocol accordingly).
- c. **Tissue.** This protocol should only be followed for use with small amounts of tissue ( $<30$  mg); if using larger amounts of tissue follow our **Large Sample Protocol**. The volume of lysis reagent should be at least 10 fold greater than the volume of tissue. Thus for  $<30$  mg of tissue use 1ml **TRIzol**. The tissue must be completely disrupted by homogenization as detailed in the **Large Sample Protocol**, using a homogenizer probe that is appropriate for small sample volume in a 2 ml microcentrifuge tube. Warning: incomplete lysis will reduce yield.

### RNA isolation using Qiagen RNeasy<sup>®</sup> Mini Columns

1. For samples processed with **TRIzol**:
  - a. Ensure that the sample is completely lysed: if working with cells or islets vortex well, or if working with tissue ensure complete homogenization. Samples can be stored at this point at  $<-70^{\circ}\text{C}$  for at least 1 year.
  - b. Incubate sample for 5 minutes in TRIzol at room temperature.
  - c. Add 0.2 ml of chloroform for every 1ml of TRIzol used. **Shake vigorously** for 15 seconds and incubate at room temperature for 2-3 min.

Note: Avoid vortexing as this may increase the DNA contamination of your RNA sample.
  - d. Centrifuge samples 5 min. at 12,000 x g at  $4^{\circ}\text{C}$ .

Note: The  $4^{\circ}\text{C}$  spins are essential for phase separation. Room temperature spins may result in variable phase separation thus resulting in variable RNA yields.
  - e. Transfer the aqueous phase to a fresh microcentrifuge tube. Proceed immediately to Step 2.

Note: The aqueous phase is the colorless upper phase that corresponds to  $\sim 60\%$  of the volume of TRIzol used. The interphase should be fairly well-defined.
2. Add **1 volume** (usually 600  $\mu$ l) of **70% ethanol** to the cleared lysate, and mix immediately by pipetting. Do not centrifuge. Continue without delay with step 3.

3. Apply up to **700 µl of the sample**, including any precipitate that may have formed, to an **RNeasy mini column** placed in a 2 ml collection tube (supplied). Close the tube gently, and **centrifuge for 15 s** at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm). Discard the flow-through, but not the collection tube.  
*Note:* If the volume exceeds 700 µl, load aliquots successively onto the RNeasy column, and centrifuge as above. Discard the flow-through after each centrifugation step.
4. Add **700 µl Buffer RW1** to the RNeasy column. Close the tube gently, and **centrifuge for 15 s** at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) to wash the column. Discard the flow-through and collection tube.
5. Transfer the RNeasy column into a new 2 ml collection tube (supplied). Pipet **500 µl Buffer RPE** onto the RNeasy column. Close the tube gently, and **centrifuge for 15 s** at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) to wash the column. Discard the flow-through, but not the collection tube.  
*Note:* Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use.
6. Add another **500 µl Buffer RPE** to the RNeasy column. Close the tube gently, and **centrifuge for 2 min** at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) to dry the RNeasy silica-gel membrane.  
*Note:* Following the centrifugation, remove the RNeasy mini column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.
7. To **elute**, transfer the RNeasy column to a new 1.5 ml collection tube (supplied). Pipet 30–50 µl **RNase-free water** directly onto the RNeasy silica-gel membrane. Close the tube gently, wait 1 min, and **centrifuge for 1 min** at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) to elute.  
*Note:* Never elute with less than 30 µl water. If the expected RNA yield is  $>30$  µg, repeat the elution step with a second volume of RNase-free water. Elute into the same collection tube.
8. Keep eluted RNA on ice at all times and **store at  $<-70^{\circ}\text{C}$** .



## Simplified RNA Isolation Protocol for Experienced Users

1. Complete sample pre-processing for samples processed in **TRIzol**:
  - a. Lyse / homogenize and incubate sample for 5 minutes in TRIzol at room temperature.
  - b. Add 0.2 ml of **chloroform** for every 1ml of TRIzol used. Shake vigorously for 15 seconds and incubate at room temperature for 2-3 min.
  - c. Centrifuge samples 5 min. at 12,000 x g at 4°C. Transfer the aqueous phase to a fresh microcentrifuge tube. Proceed immediately to Step 2.
2. Add **1 volume** (usually 600 µl) of **70% ethanol** to the cleared lysate, and mix immediately by pipetting.
3. Apply **up to 700 µl of the sample** to an **RNeasy mini column** placed in a 2 ml collection tube. Centrifuge for 15s and discard the flow-through. Repeat as necessary.
4. Add **700 µl Buffer RW1** to the RNeasy column. Centrifuge for 15 s as above.
5. Transfer the RNeasy column into a new 2 ml collection tube. Pipet **500 µl Buffer RPE** onto the RNeasy column. Centrifuge as above and discard the flow-through.
6. Add another 500 µl Buffer RPE to the RNeasy column. Centrifuge for 2 min to dry the RNeasy silica-gel membrane.
7. To elute, transfer the RNeasy column to a new 1.5 ml collection tube. Pipet 30–50 µl **RNase-free water** directly onto the RNeasy silica-gel membrane. Wait 1 min, and centrifuge for 1 min to elute.
8. Keep eluted RNA on ice at all times and **store at <-70°C**.