RNA Extraction from Mammalian Tissue – Traditional Method

Reagents

Guanidine thiocyanate (GTC): Sigma (G9277-500G).

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

1 M Trizma® hydrochloride buffer solution (Tris), pH 7.5: Sigma (T2319-1L).

2-Mercaptoethanol: Sigma (M3148-100ML)

GTC Stock Solution (500 ml):

<table>
<thead>
<tr>
<th></th>
<th>Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanidine thiocyanate</td>
<td>236 g</td>
</tr>
<tr>
<td>1 M Trizma® HCl, pH 7.5</td>
<td>50 ml</td>
</tr>
</tbody>
</table>

Dissolve GTC with Trizma buffer and about 300 ml of DEPC-treated H₂O. Add a magnetic bar and stir on a combination heater-stirrer at 65 °C (do not exceed this) until all the GTC is dissolved. Cool the solution and adjust the volume to 500 ml. The solution is stable for months as room temperature is light sensitive so wrap the storage bottle in foil.

Solution D (Denaturing Homogenization Buffer): Working in a fume hood, immediately prior to use to the GTC stock solution add 2-Mercaptoethanol to a final concentration of 1% (0.14M). e.g. to 49.5 ml of GTC stock solution add 0.5 ml of 14.3 M 2-Mercaptoethanol.

Acid-Phenol:Chloroform, 5:1, pH 4.5 (premixed with IAA (125:24:1)): Ambion (Cat. No. 9722). Stable for 6 months if stored at 4°C. Phenol extraction is a commonly used method for deproteinization of nucleic acids. Most proteins are more soluble in phenol than in the aqueous phase. Conversely, nucleic acids are more soluble in the aqueous phase. Centrifugation of the mixture will yield two phases, the lower phase is the organic phase and will contain the protein, usually as a white flocculent at the interface. The upper aqueous phase will contain nucleic acids. Phase partitioning of nucleic acids is also pH dependent. At pH 4-6 DNA will be retained in the organic phase and interface, leaving the RNA in the aqueous phase. Isolation of RNA from biological material is often done with an acid-phenol. For DNA isolation a pH of 7.5-8.0 is required and both DNA and RNA will partition into the upper aqueous phase. Chloroform is mixed with phenol to increase the efficiency of nucleic acid extractions. The increased efficiency of extraction is due to chloroform's ability to denature proteins, thus aiding separation of nucleic acid from protein. Phase separation of the extracted solution is also enhanced, thereby assisting in the removal of the aqueous phase with minimal cross contamination from the organic phase. Addition of chloroform to the phenol also aids in the removal of lipids. Often isoamyl alcohol is added to the phenol:chloroform to prevent foaming.

3 M Sodium Acetate, pH 5.5: Ambion (Cat. No. 9740).

Phenol:Chloroform:IAA, 25:24:1, pH 6.6: Ambion (Cat. No. 9732). Stable for 6 months if stored at 4°C. Do not add additional buffer if used for RNA extraction.

2-Propanol (isopropanol): Fisher Scientific (Cat. No. A416-4)

70% Ethanol (in RNase-free H₂O)

For regular RNA extraction 70-75% Ethanol is recommended for washing steps. However, for low nucleic acids amounts 90-95% Ethanol is recommended.
RNA storage buffers. 3 ideal qualities of a storage buffer are that it be RNase-free, have a low pH (pH 6-7), and incorporate a chelating agent to protect against RNA degradation by introduced RNases:

- **THE RNA Storage Solution**: Ambion (Cat. No. 7001). 1 mM sodium citrate, pH 6.4. This solution has two features which minimize base hydrolysis of RNA: a low pH, and sodium citrate, which is an efficient chelating agent. It is compatible with all common RNA applications such as reverse transcription, in vitro transcription, Northern analysis and nuclease protection assays.
- **TE Buffer (10 mM Tris-HCl, 1 mM EDTA), pH 7.0**: Ambion (Cat. No. 9861).
- **0.1mM EDTA, pH 8.0**: Ambion (Cat. No. 9912)

100 % (200 proof) Ethanol: Pharmco (Cat. No. 111ACS200)
50 ml Falcon Tubes (BD Cat. No. 352070). 9400 RCF rating.
15 ml Falcon Tubes (BD Cat. No. 352097). 6000 RCF rating.

Protocol

Preparation

Clean the homogenizer probe by running it at maximum speed in the probe wash tubes (50 ml conical tubes) as follows:

1. RNAse ZAP™: 30s
2. DEPC Water: 30s
3. 100% Ethanol: 30s
4. DEPC Water: 30s

Sample preparation and homogenization

1. Arrange appropriately labeled 50 ml conical tubes with 10 ml of **denaturing solution** in each on ice.
2. Quickly dissect out up to 100 mg to 1 g of tissue and place immediately into tubes containing **cold denaturing solution** on ice.

*Note 1*: It is essential that after the tissue is homogenized in the solution the final concentration of GTC is > 3 M to inactivate endogenous RNases. If the volume of the material is sufficiently large it is possible that the GTC will be diluted to the point where the concentration falls below 3 M and RNases become active.

*Note 2*: If working the pancreas, the animal should be anesthetized and the pancreas removed while the animal is still living - immediately proceed to step 2, before processing any other samples.

3. Immediately homogenize the tissue using a conventional rotor–stator homogenizer for at least 45 s at maximum speed until the sample is uniformly homogeneous.
4. Place homogenate on ice. Alternatively, snap freeze the homogenate in liquid nitrogen and store at -80°C for future RNA extraction.
5. Wash the homogenizer probe as above and repeat steps 2 to 4 for each sample. When finished, ensure that the probe is thoroughly cleaned.
**RNA isolation using traditional Phenol:Chloroform Extraction**

1. Add an equal volume (~11 ml) of Phenol:Chloroform:IAA (25:24:1). Be sure to use the organic phase of the Phenol:Chloroform:IAA which lies under the thin upper layer of aqueous buffer.

2. Shake or vortex vigorously for 1 min. Incubate on ice for 15 min. This step ensures the complete disassociation of nucleoprotein complexes.

3. Centrifuge at $\geq 10,000 \times g$ (8000 rpm in Sorval SLA-600TC rotor) for 15 min, preferably at 4°C.

4. Without disturbing the interface, carefully transfer the aqueous phase (~11 ml) to a fresh tube.

5. Add 1/10 aqueous phase volume (~1.1 ml) of 3 M Sodium Acetate to the phenol extracted homogenate. Mix by shaking or inversion for about 10 sec.

6. Add 1 aqueous phase volume (~11 ml) of Acid-Phenol:Chloroform (5:1). Be sure to use the organic phase of the Phenol:Chloroform:IAA which lies under the thin upper layer of aqueous buffer.

7. Shake or vortex vigorously for 1 min. Incubate on ice for 15 min. This step ensures the complete disassociation of nucleoprotein complexes.

8. Centrifuge at $\geq 10,000 \times g$ for 15 min, preferably at 4°C.

9. Without disturbing the interface, carefully transfer the aqueous phase (~11 ml) to a fresh tube.

10. Precipitate the RNA by adding an equal volume (~11 ml) of 100% isopropanol. Invert tube to mix and incubate at -20°C for at least 1 hour.

11. Centrifuge at $\geq 10,000 \times g$ for 20 min at 4°C to pellet the RNA.

12. Carefully remove the supernatant solution and wash the pellet with 5 ml of cold 70% ethanol by vortexing.

13. Centrifuge at low speed (~3,000 x g - 4000 rpm in Sorval SLA-600TC rotor) for 5 min at room temperature or 4°C. Remove the supernatant and pulse spin to bring down residual ethanol. Remove residual ethanol with a fine-tipped pipette and allow the pellet to air dry for 1 to 5 min. Do not let the RNA pellet dry completely as this will greatly decrease its solubility.

14. Resuspend the RNA in an appropriate amount (100 to 1000 µl) of RNase-free buffer, water, or in formamide. Briefly vortex or repeatedly pipette to aid in resuspension, and if necessary, heat to ~60°C. A freeze-thaw cycle may also enhance resuspension.

15. Store at -80°C.