Purification des ARN totaux de cellules animales :

1. Add 500 μl Guanidium Solution at 50 mg of tissue and homogenize it at 4°C in a sterile microcentrifuge tube using a pestle homogenizer until a smooth, homogenous suspension is obtained (see Hint #4).

2. Add 50 μl of 2 M Sodium Acetate pH 4.0 and mix vigorously.

3. Add 500 μl of Phenol and vortex sample.

4. Add 100 μl of Chloroform and vortex sample.

5. Incubate on ice for 15 min.

6. Centrifuge the mixture in microcentrifuge at maximum speed for 10 min at 4°C.

7. Transfer the upper, aqueous phase to a sterile microcentrifuge tube.

8. Repeat Steps #3 through #7 until the interface between the phases is clear.

9. After transferring the aqueous phase into a fresh sterile tube, add 500 μl of Isopropanol and vortex to mix.

10. Incubate at -20°C for 20 min.

11. Centrifuge the mixture in a microcentrifuge at maximum speed for 10 min to pellet the RNA.

12. Decant the liquid carefully, add 500 μl of 70% Ethanol to the pellet and mix by vortexing to precipitate the RNA.

13. Centrifuge the RNA sample in microcentrifuge at maximum speed for 10 min at 4°C.

14. Remove all of the liquid and air-dry RNA pellet.

15. Dissolve the dry RNA pellet in 200 μl of 0.5% SDS (DEPC-treated) at 65°C in a heating block or a water bath.


17. Centrifuge the mixture in microcentrifuge at maximum speed for 10 min at 4°C.

18. Transfer the upper, aqueous phase to a sterile microcentrifuge tube.

19. Repeat Steps #16 through #18 until the interface between the phases is clear.

20. After transferring the aqueous phase to a fresh microcentrifuge tube, add 20 μl of Acetate Buffer, then 500 μl of Ethanol to precipitate the RNA.

21. Incubate at -20°C for 20 min.
22. Pellet the RNA by centrifuging the tube at maximum speed in a microcentrifuge for 10 min.

23. Decant the liquid and add 500 μl of 70% Ethanol to the pellet and vortex to mix.

24. Centrifuge in a microcentrifuge at maximum speed for 10 min to pellet the RNA.

25. Remove all the liquid and allow the pellet to air dry.

26. The pellet can be reconstituted in a desired volume of DEPC-treated TE Buffer if further enzymatic manipulation of RNA is required. For prolonged storage, RNA may be dissolved in DEPC-treated 0.5% SDS to inhibit ribonucleases activity.

<table>
<thead>
<tr>
<th>Phenol:Chloroform</th>
<th>25:24:1 Phenol:Chloroform:Isoamyl Alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>TE Buffer</td>
<td>Make up in DEPC-treated ddH₂O</td>
</tr>
<tr>
<td></td>
<td>10 mM Tris</td>
</tr>
<tr>
<td></td>
<td>pH 8.0</td>
</tr>
<tr>
<td></td>
<td>1 mM EDTA</td>
</tr>
<tr>
<td>DEPC-treated ddH₂O (Diethyl pyrocarbonate)</td>
<td>0.1 % v/v</td>
</tr>
<tr>
<td>Guanidine Solution</td>
<td>4 M Guanidine Isothiocyanate + 0.5% (w/v) Sarkosyl</td>
</tr>
<tr>
<td></td>
<td>+ 25 mM Sodium Citrate</td>
</tr>
<tr>
<td></td>
<td>pH 7.0</td>
</tr>
<tr>
<td></td>
<td>(ajouter du 2-Mercaptoethanol 100 mM final avant utilisation) Stocker à 4°C</td>
</tr>
<tr>
<td>0.5% (v/v) SDS</td>
<td>Vérifier stock à 10 % m/v</td>
</tr>
<tr>
<td>70% (v/v) Ethanol</td>
<td>Vérifier stock à –20° en salle 008</td>
</tr>
<tr>
<td>DEPC-Treated Acetate Buffer</td>
<td>0.1 M Magnesium Acetate + 3 M Sodium Acetate</td>
</tr>
<tr>
<td></td>
<td>pH 5.2</td>
</tr>
<tr>
<td>2 M Sodium Acetate, pH 4.0</td>
<td></td>
</tr>
<tr>
<td>1 M Sodium Citrate, pH 7.0</td>
<td></td>
</tr>
</tbody>
</table>

1. CAUTION! This substance is a biohazard. Consult this agent's MSDS for proper handling instructions.

2. This method is recommended for purification of RNA from 50 mg of starting material. Appropriate scaling up of the reagent's volumes should be used in order to accommodate larger quantities.

3. After centrifugation, RNA is present in the aqueous phase, while, due to protonation at the acidic pH used, genomic DNA is partitioned into the Phenol phase.

4. RNase-Free Conditions for RNA Work

To minimize degradation of RNA by RNases, wear gloves when handling samples and reagents and change gloves regularly while working. Treat water and solutions with DEPC* (Diethyl Pyrocarbonate) to inactive RNases and use solutions prepared with RNase-free water and equipment. Use sterile plasticware or glassware that have been baked at 150°C in dry heat for at least 2 hr. Autoclaving solutions and vessels is not sufficient to remove all RNase activity. Do not use spatulas to measure out chemicals used for RNase-free solutions. Reserve RNase-free solutions, reagents, and consumables, such as pipette tips, only for RNA work.

Some researchers find that following all of the above measures is not necessary for good quality RNA preps. You must determine for your own work how conservative to be in eliminating sources of contaminating RNases.

* To treat water with DEPC

Add DEPC to ddH₂O to a final concentration of 0.1% (v/v). Shake up or stir solution. Allow solution to sit for at least 12 hr. Autoclave for 15 min to inactivate the DEPC.
Vérification de la pureté et de l’état de dégradation des ARN par électrophorèse

Préparer des gels à 1% en agarose en tampon TBE 0,5 X.
Mesurer Abs 260, spectre (en cuve ...)
Déposer 1 et 5 µg d’ARN dénaturé ou pas par la chaleur (1 min 95 °C) puis trempé.
Marqueur de taille ARN (absent)

Sources :


Compte-rendu

1) Expliquer le rôle (moléculaire) de chaque étape (et produits) :
   - quel est le pH de la solution à l’étape 2, que deviennent les différents types d’acides nucléiques ;
   - les conditions pour précipiter les ARN sont elles réunies ?

2) Légendier la photo de l’électrophorèse, conclure.

![Analysis of Total RNA](image)

*Figure 1.* Formaldehyde agarose gel of total RNA isolated from the indicated sources using RNeasy kits. 10 µg RNA was loaded per lane.