Protocol for Isolation of RNA from Non-Fibrous Tissue


2. Verify that 1-Thioglycerol has been added to the LBA Buffer. Add LBA + TG Buffer to the tissue sample in accordance with the table below.

<table>
<thead>
<tr>
<th>Tissue Input Range</th>
<th>LBA + TG Buffer</th>
<th>100% Isopropanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤5mg</td>
<td>250µl</td>
<td>85µl</td>
</tr>
<tr>
<td>&gt;5mg</td>
<td>500µl</td>
<td>170µl</td>
</tr>
</tbody>
</table>

3. Disrupt up to 20mg of sample using a tissue homogenizer, followed by pipetting 7–10 times to shear the DNA using a P200 or P1000 pipettor.

4. Clear homogenates by centrifugation for 3 minutes at 14,000 × g, then transfer them to a clean tube.

5. Add Isopropanol as recommended in the table above. Mix by vortexing 5 seconds.

6. Wearing gloves, unpack one Minicolumn, two Collection Tubes and one Elution Tube for each sample. Label each tube and Minicolumn. Place one Minicolumn into a Collection Tube for each sample.

7. Transfer lysate to a Minicolumn in a Collection Tube. Centrifuge at 12,000–14,000 × g for 1 minute at 20–25°C.

8. Remove the ReliaPrep™ Minicolumn, and discard liquid in the Collection Tube. Replace the Minicolumn in the Collection Tube. Add 500µl of RNA Wash Solution to the Minicolumn. Centrifuge at 12,000–14,000 × g for 30 seconds. Empty the Collection Tube, and place it in the microcentrifuge rack.


10. Add Column Wash Solution. Centrifuge for 15 seconds.


12. Transfer the Minicolumn to a new Collection Tube. Add RNA Wash Solution. Centrifuge for 2 minutes.


14. Store the Elution Tube with RNA at –70°C.
Protocol for the Isolation of RNA from Non-Fibrous Tissue (continued)

9. Prepare DNase I incubation mix by combining the following amounts of reagent, per sample, in the order listed:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Volume</th>
<th>Number of Preps</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow Core Buffer</td>
<td>24µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MnCl₂, 0.09M</td>
<td>3µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNase I</td>
<td>3µl</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix by gently pipetting; do not vortex. The volumes listed above make enough DNase I mix for a single sample. Multiply this amount by the number of samples to calculate the amount of DNase I mix to prepare.

10. Apply 30µl of DNase I incubation mix to the Minicolumn membrane. Incubate for 15 minutes at 20°–25°C.

11. Add 200µl of Column Wash Solution (with ethanol added) to the Minicolumn. Centrifuge at 12,000–14,000 × g for 15 seconds.

12. Add 500µl of RNA Wash Solution (with ethanol added). Centrifuge at 12,000–14,000 × g for 30 seconds. Discard the wash solutions and the Collection Tube.

13. Place the ReliaPrep™ Minicolumn into a new Collection Tube. Add 300µl of RNA Wash Solution and centrifuge at high speed for 2 minutes.

14. Transfer the ReliaPrep™ Minicolumn from the Collection Tube to an Elution Tube. Add Nuclease-Free Water to the Minicolumn membrane as recommended in the table below. Place the Minicolumn and Elution Tube into a centrifuge with the Elution Tube lid facing to the outside. Centrifuge at 12,000–14,000 × g for 1 minute.

<table>
<thead>
<tr>
<th>Tissue Input Range</th>
<th>Nuclease-Free Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>5mg or less</td>
<td>15µl</td>
</tr>
<tr>
<td>5 to 20mg</td>
<td>30µl</td>
</tr>
</tbody>
</table>

15. Discard the Minicolumn. Cap the Elution Tube containing the purified RNA and store at −70°C.

Protocol for Isolation of RNA from Fibrous Tissue


2. Verify that 1-Thioglycerol has been added to the LBA Buffer. Add LBA + TG Buffer to the tissue sample in accordance with the table below.

<table>
<thead>
<tr>
<th>Tissue Input Range</th>
<th>LBA + TG Buffer</th>
<th>RDB Addition (µl)</th>
<th>Total Volume (µl)</th>
<th>100% Isopropanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤5mg</td>
<td>250µl</td>
<td>250µl</td>
<td>500µl</td>
<td>170µl</td>
</tr>
<tr>
<td>&gt;5mg</td>
<td>500µl</td>
<td>500µl</td>
<td>1,000µl</td>
<td>340µl</td>
</tr>
</tbody>
</table>

3. Disrupt up to 20mg of sample using a tissue homogenizer, followed by pipetting 7–10 times to shear the DNA using a P200 or P1000 pipettor.

4. Add an equal volume of RNA Dilution Buffer (RDB) and mix by vortexing for 10 seconds. Incubate 1 minute at room temperature. A visible precipitate may appear. Clear homogenates by centrifuging 3 minutes at 10,000 × g. Transfer cleared lysates to clean tubes.

5. Add isopropanol as recommended in the table above. Mix by vortexing for 5 seconds.

6. Wearing gloves, unpack one Minicolumn, two Collection Tubes and one Elution Tube for each sample. Label each tube and Minicolumn. Place one Minicolumn into a Collection Tube for each sample.

7. Transfer lysate to a Minicolumn in a Collection Tube. Centrifuge at 12,000–14,000 × g for 1 minute seconds at 20–25°C.

   **Note:** If 500µl of LBA + TG Buffer is used for homogenizing, you will need to transfer the lysate in two 670µl aliquots, centrifuging after each transfer.

8. Remove the ReliaPrep™ Minicolumn, and discard liquid in the Collection Tube. Replace the Minicolumn in the Collection Tube. Add 500µl of RNA Wash Solution to the Minicolumn. Centrifuge at 12,000–14,000 × g for 30 seconds. Empty the Collection Tube, and place it in the microcentrifuge rack.


10. Store the Elution Tube with RNA at −70°C.
Protocol for Isolation of RNA from Fibrous Tissue (continued)

9. Prepare DNase I incubation mix by combining the following amounts of reagent, per sample, in the order listed:

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Mix by gently pipetting; do not vortex. The volumes listed above make enough DNase I mix for a single sample. Multiply this amount by the number of samples to calculate the amount of DNase I mix to prepare.

10. Apply 30µl of DNase I incubation mix to the Minicolumn membrane. Incubate for 15 minutes at 20°–25°C.

11. Add 200µl of Column Wash Solution (with ethanol added) to the Minicolumn. Centrifuge at 12,000–14,000 × g for 15 seconds.

12. Add 500µl of RNA Wash Solution (with ethanol added). Centrifuge at 12,000–14,000 × g for 30 seconds. Discard the wash solutions and the Collection Tube.

13. Place the ReliaPrep™ Minicolumn into a new Collection Tube. Add 300µl of RNA Wash Solution and centrifuge at high speed for 2 minutes.

14. Transfer the ReliaPrep™ Minicolumn from the Collection Tube to an Elution Tube. Add Nuclease-Free Water to the Minicolumn membrane as recommended in the table below. Place the Minicolumn and Elution Tube into a centrifuge with the Elution Tube lid facing to the outside. Centrifuge at 12,000–14,000 × g for 1 minute.

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15. Discard the Minicolumn. Cap the Elution Tube containing the purified RNA and store at −70°C.