ReliaPrep™ RNA Tissue Miniprep System

INSTRUCTIONS FOR USE OF PRODUCTS Z6110, Z6111 AND Z6112.

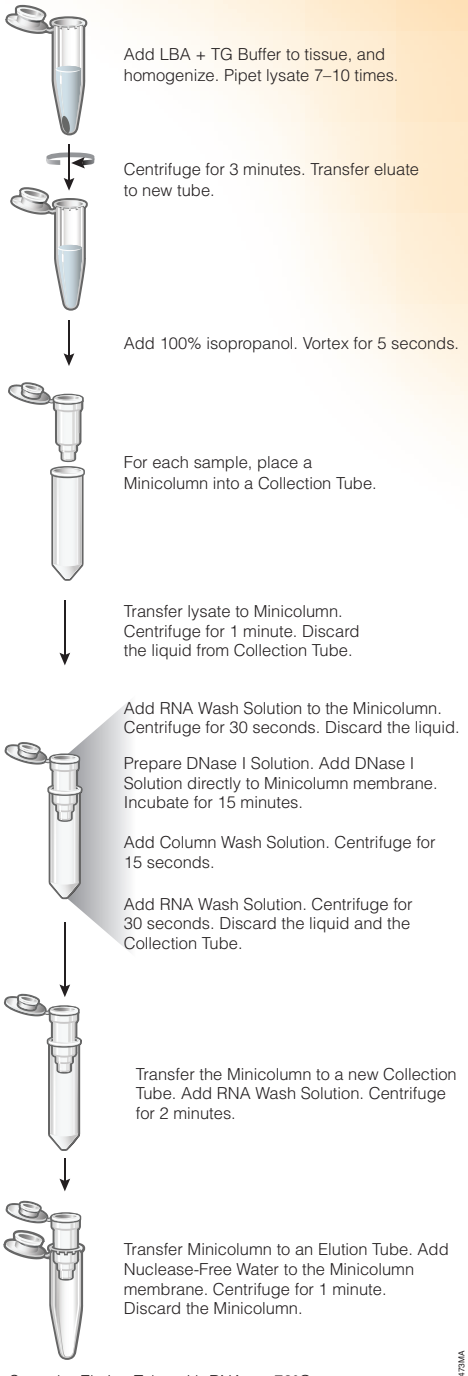
Quick PROTOCOL

Protocol for Isolation of RNA from Non-Fibrous Tissue

1. Before beginning the ReliaPrep™ RNA Tissue Miniprep protocol, see **Section 4.A., Preparation of Solutions**, in the *ReliaPrep™ RNA Tissue Miniprep System Technical Manual*, #TM394. For best results, Prepare the four required solutions immediately prior to use.
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.

Tissue Input Range	LBA + TG Buffer	100% Isopropanol
≤5mg	250µl	85µl
>5mg	500µl	170µl

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.



Store the Elution Tube with RNA at –70°C.

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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:*

Solution	Volume	×	Number of Preps	= Total
Yellow Core Buffer	24µl			
$MnCl_2$, 0.09M	3µl			
DNase I	3µl			

   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.

Tissue Input Range	Nuclease-Free Water
5mg or less	15µl
5 to 20mg	30µl

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

Detailed protocol information is available in the *ReliaPrep™ RNA Tissue Miniprep System Technical Manual* #TM394, available at:
www.promega.com/protocols/

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INSTRUCTIONS FOR USE OF PRODUCTS Z6110, Z6111 AND Z6112.

Quick PROTOCOL

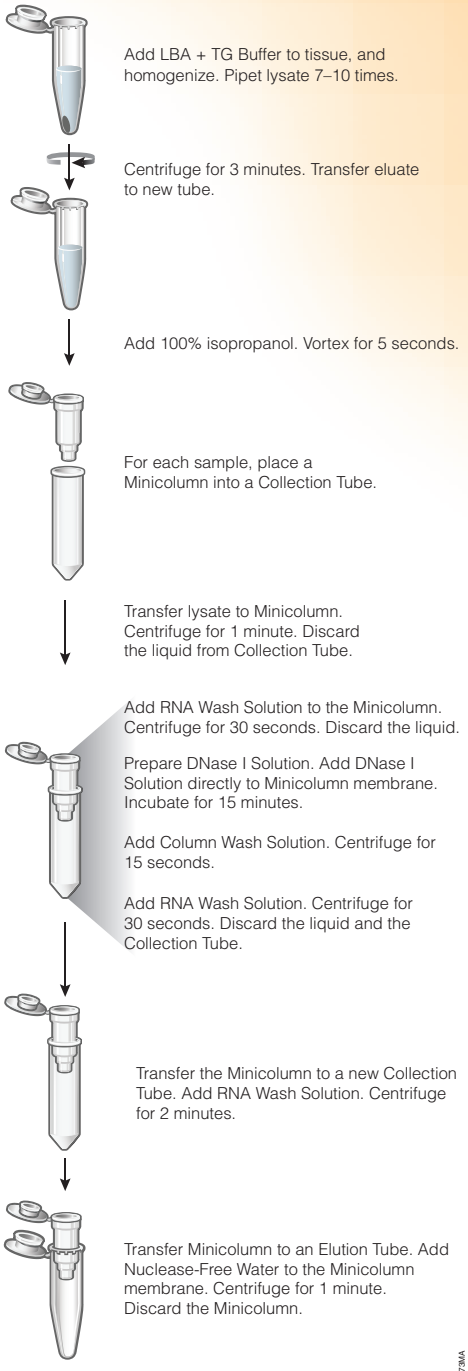
Protocol for Isolation of RNA from Fibrous Tissue

1. Before beginning the ReliaPrep™ RNA Tissue Miniprep protocol, see **Section 4.A., Preparation of Solutions**, in the *ReliaPrep™ RNA Tissue Miniprep System Technical Manual*, #TM394. For best results, Prepare the four required solutions immediately prior to use.
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.

Tissue Input Range	LBA + TG Buffer	RDB Addition (µl)	Total Volume (µl)	100% Isopropanol
≤5mg	250µl	250µl	500µl	170µl
>5mg	500µl	500µl	1,000µl	340µl

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.



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Protocol for Isolation of RNA from Fibrous Tissue (continued)

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

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