

ReliaPrep™ RNA Miniprep System for Tissues

Instructions for Use of Products **Z6014** and **Z6015**.

Protocol for Isolating RNA from Non-Fibrous Tissues

- Before beginning the ReliaPrep™ RNA Miniprep for Tissues protocol, see **Section 4.A., Preparing Solutions**, in the *ReliaPrep™ RNA Miniprep System Technical Manual #TM774*. For best results, prepare the four required solutions immediately prior to use.
- 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.

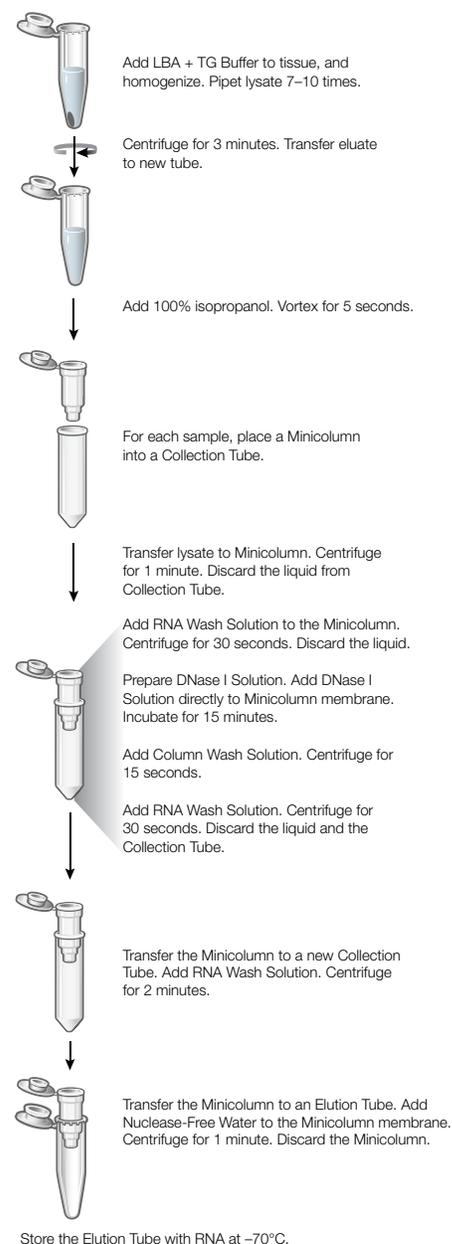
Tissue Input Range	LBA + TG Buffer	100% Isopropanol
≤5mg	250µl	85µl
>5mg	500µl	170µl

- 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.
- Clear homogenates by centrifugation for 3 minutes at 14,000 × g, then transfer them to a clean tube.
- Add Isopropanol as recommended in the table above. Mix by vortexing 5 seconds.
- 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.
- Transfer lysate to a Minicolumn in a Collection Tube. Centrifuge at 12,000–14,000 × g for 1 minute at 20–25°C.
- 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.
- 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.

- Apply **30µl of DNase I incubation mix** to the Minicolumn membrane. Incubate for 15 minutes at 20–25°C.
- Add **200µl of Column Wash Solution** (with ethanol added) to the Minicolumn. Centrifuge at 12,000–14,000 × g for 15 seconds.
- 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.



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Protocol for Isolating RNA from Non-Fibrous Tissues (continued)

- Place the ReliaPrep™ Minicolumn into a new Collection Tube. Add **300µl** of **RNA Wash Solution** and centrifuge at high speed for 2 minutes.
- 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	15µl
5–20mg	30µl

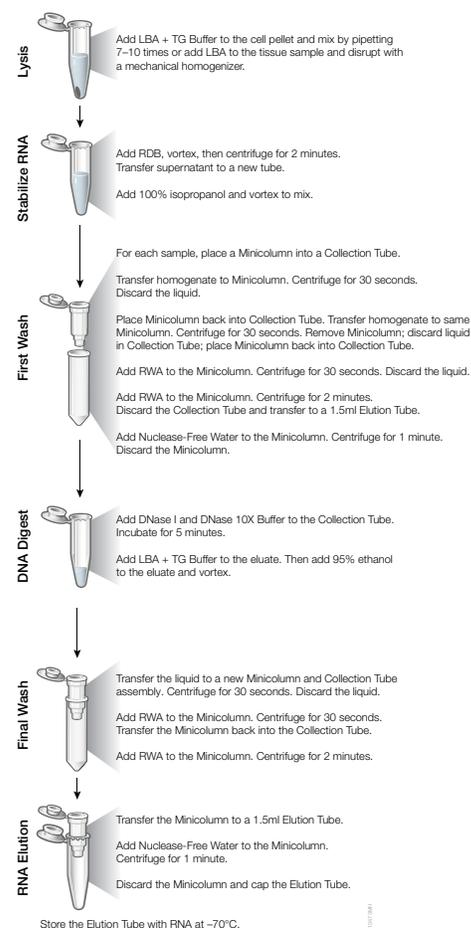
- Discard the Minicolumn. Cap the Elution Tube containing the purified RNA and store at –70°C.

Protocol for Isolating RNA from Fibrous Tissues

- Before beginning the ReliaPrep™ RNA Miniprep for Tissues protocol, see **Section 4.A., Preparing Solutions**, in the *ReliaPrep™ RNA Miniprep System Technical Manual #TM774*. For best results, prepare the four required solutions immediately prior to use.
- 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

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



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Protocol for Isolating RNA from Fibrous Tissues (continued)

- 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.
- 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.
- 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.

- Apply **30µl of DNase I incubation mix** to the Minicolumn membrane. Incubate for 15 minutes at 20–25°C.
- Add **200µl of Column Wash Solution** (with ethanol added) to the Minicolumn. Centrifuge at 12,000–14,000 × *g* for 15 seconds.
- 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.
- Place the ReliaPrep™ Minicolumn into a new Collection Tube. Add **300µl of RNA Wash Solution** and centrifuge at high speed for 2 minutes.
- 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	15µl
5–20mg	30µl

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

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Additional protocol information is in Technical Manual #TM774, available online at: www.promega.com