

# Total RNA Mini Kit (Tissue)

*For research use only*

<b>Sample</b>	: up to 25 mg of tissue, up to 25 mg of paraffin-embedded tissue
<b>Yield</b>	: 5-30 µg
<b>Format</b>	: spin column
<b>Operation time</b>	: within 60 minutes

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## Introduction

The Total RNA Mini Kit (Tissue) was designed specifically for purifying total RNA from a variety of animal and paraffin-embedded tissue. Tissue samples can be efficiently homogenized in a microcentrifuge tube using the provided micropestle. Detergents and chaotropic salt are used to lyse cells and inactivate RNase and optional DNase treatments can be followed to remove unwanted DNA residue. RNA in the chaotropic salt is bound by the glass fiber matrix of the spin column (1). Once any contaminants have been removed, using the Wash Buffer (containing ethanol), the purified total RNA is eluted by RNase-free water, and is ready for use in RT-PCR, Northern Blotting, Primer Extension and cDNA Library Construction. Phenol extraction or alcohol precipitation is not required.

## Quality Control

The quality of the Total RNA Mini Kit (Tissue) is tested on a lot-to-lot basis by isolating total RNA from a 25 mg animal tissue sample. The purified RNA is quantified with a spectrophotometer and checked by electrophoresis.

### Kit Contents

Name	RT004	RT050	RT100	RT300
RB Buffer	2 ml	30 ml	60 ml	130 ml
W1 Buffer	2 ml	30 ml	50 ml	130 ml
Wash Buffer <sup>1</sup> (Add Ethanol)	1 ml (4 ml)	12.5 ml (50 ml)	25 ml (100 ml)	50 ml x 2 (200 ml) x 2
RNase-free water	1 ml	6 ml	15 ml	30 ml
RB Column	4 pcs	50 pcs	100 pcs	300 pcs
Filter Column	4 pcs	50 pcs	100 pcs	300 pcs
2 ml Collection Tube	8 pcs	100 pcs	200 pcs	600 pcs
Micropestle	4 pcs	50 pcs	100 pcs	300 pcs

### Order Information

Product Name	Package size	Cat. No.
Total RNA Mini Kit (Blood/Cultured Cell)	50/100/300 preps	RB050/100/300
Total RNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	RBM10/25
Total RNA Mini Kit (Tissue)	50/100/300 preps	RT050/100/300
Total RNA Maxi Kit (Tissue)	10/25 preps	RTM10/25
Total RNA Mini Kit (Plant)	50/100/300 preps	RP050/100/300
Total RNA Maxi Kit (Plant)	10/25 preps	RPM10/25
miRNA Isolation Kit	100 preps	PU009100
96-Well Total RNA Kit	2/4/10 x 96 Wells	RBP02/04/10

<sup>1</sup>Add absolute ethanol to the Wash Buffer prior to initial use (see bottle label for volume).

## Caution

RB Buffer contains chaotropic salt, which is a harmful irritant. During operation, always wear a lab coat, disposable gloves, and protective goggles.

## References

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615

## Total RNA Mini Kit (Tissue) Protocol

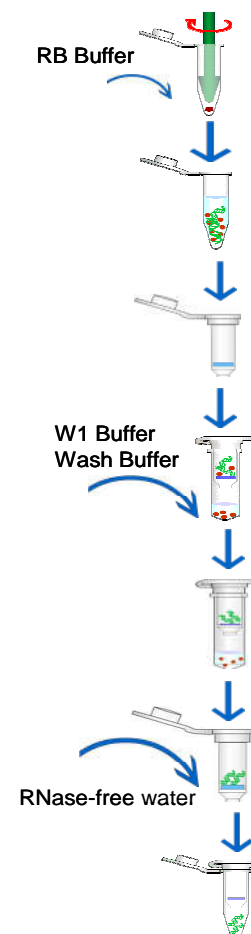
- Add absolute ethanol to the Wash Buffer prior to initial use (see bottle label for volume).
- Additional requirements: 20-G needle syringe, absolute ethanol, microcentrifuge tubes (RNase free),  $\beta$ -mercaptoethanol

### DNA Residue Degradation options:

► Optional Step 1 (DNA Residue Degradation): Add 100  $\mu$ l of DNase I (2 KU/ml) mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM  $MnCl_2$ , 50  $\mu$ g/ml BSA at 25°C} to the center of the RB Column matrix. Let stand for 10 minutes at room temperature and then proceed to Step 4 Wash.

► Optional Step 2 (DNA Residue Degradation): Add 2  $\mu$ l of DNase I (2 KU/ml) mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM  $MnCl_2$ , 50  $\mu$ g/ml BSA at 25°C} to the final elution sample. Let stand for 10 minutes at room temperature.

Step 1 Cell Lysis	<ul style="list-style-type: none"> <li>➤ Cut off up to 25 mg of fresh or frozen animal tissue and transfer it to a 1.5 ml microcentrifuge tube (If using frozen animal tissue, the sample MUST have been flash frozen in liquid nitrogen and immediately stored at -70°C until use, to avoid RNA Degradation).</li> <li>➤ Add <b>400 <math>\mu</math>l of RB Buffer</b> and 4 <math>\mu</math>l of <math>\beta</math>-mercaptoethanol to the tube and use the provided <b>Micropestle</b> to grind the tissue a few times.</li> <li>➤ Shear the tissue by passing lysate through a 20-G needle syringe 10 times.</li> <li>➤ Incubate at room temperature for 5 minutes.</li> <li>➤ Place a <b>Filter Column</b> in a <b>2 ml Collection Tube</b> and transfer the sample mixture to the <b>Filter Column</b>.</li> <li>➤ Centrifuge for 1 minute at 1,000 x g.</li> <li>➤ Discard the <b>Filter Column</b> and proceed to Step 2 RNA Binding.</li> </ul>
Step 2 RNA Binding	<ul style="list-style-type: none"> <li>➤ Add 400 <math>\mu</math>l of 70% ethanol to the filtrate from Step 1 and shake vigorously (break up any precipitate with pipetting).</li> <li>➤ Place a <b>RB Column</b> in a <b>2 ml Collection tube</b>.</li> <li>➤ Transfer the ethanol added mixture to the <b>RB Column</b>.</li> <li>➤ Centrifuge at 14-16,000 x g for 2 minutes (if the lysate mixture could not flow past the RB Column membrane following centrifugation, increase the centrifuge time until the lysate mixture passes completely).</li> <li>➤ Discard the flow-through and place the <b>RB Column</b> back in the <b>2 ml Collection Tube</b>.</li> </ul> <p>Optional Step 1: DNA Residue Degradation (see options above)</p>
Step 3 Wash	<ul style="list-style-type: none"> <li>➤ Add <b>400 <math>\mu</math>l of W1 Buffer</b> to the <b>RB Column</b>.</li> <li>➤ Centrifuge at 14-16,000 x g for 1 minute.</li> <li>➤ Discard the flow-through and place the <b>RB Column</b> back in the <b>2 ml Collection Tube</b>.</li> <li>➤ Add <b>600 <math>\mu</math>l of Wash Buffer</b> (ethanol added) into the <b>RB Column</b>.</li> <li>➤ Centrifuge at 14-16,000 x g for 1 minute.</li> <li>➤ Discard the flow-through and place the <b>RB Column</b> back in the <b>2 ml Collection Tube</b>.</li> <li>➤ Add <b>600 <math>\mu</math>l of Wash Buffer</b> (ethanol added) into the <b>RB Column</b>.</li> <li>➤ Centrifuge at 14-16,000 x g for 1 minute.</li> <li>➤ Discard the flow-through and place the <b>RB Column</b> back in the <b>2 ml Collection Tube</b>.</li> <li>➤ Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.</li> </ul>
Step 4 RNA Elution	<ul style="list-style-type: none"> <li>➤ Place the dried <b>RB Column</b> in a clean 1.5 ml microcentrifuge tube (RNase-free).</li> <li>➤ Add <b>50 <math>\mu</math>l of RNase-free water</b> to the center of the column matrix.</li> <li>➤ Let stand for 3 minutes or until the water is absorbed by the matrix.</li> <li>➤ Centrifuge at 14-16,000 x g for 1 minute to elute the purified RNA.</li> </ul> <p>Optional Step 2: DNA Residue Degradation (see options above)</p>



## Total RNA Mini Kit (Tissue) Paraffin-Embedded Tissue Protocol

- Additional Requirements: xylene, absolute ethanol, microcentrifuge tube

Sample Preparation	<ul style="list-style-type: none"><li>● Slice small sections (up to 25 mg) from blocks of paraffin-embedded tissue and transfer to a 1.5 ml microcentrifuge tube.</li><li>● Add 1 ml of xylene to the tube. Vortex vigorously and incubate at room temperature for approximately 10 minutes. Vortex occasionally during incubation.</li><li>● Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.</li><li>● Add 1 ml of absolute ethanol to wash the sample pellet and mix by inverting.</li><li>● Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.</li><li>● Add 1 ml of absolute ethanol to wash the sample pellet again and mix by inverting.</li><li>● Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.</li><li>● Open the tube and incubate at 37°C for 15 minutes to evaporate any ethanol residue.</li><li>● Proceed with the Lysis Step of the Tissue Protocol on page 2.</li></ul>
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## Troubleshooting

Problem	Possible Reasons/Solution
Clogged RNA Spin Column	<ul style="list-style-type: none"><li>● Inefficient disruption and/or homogenization</li><li>● Too much starting material</li><li>● Centrifugation temperature was too low (should be 20-25°C)</li></ul>
Low RNA Yield	<ul style="list-style-type: none"><li>● Insufficient disruption and homogenization</li><li>● Too much starting material</li><li>● RNA still bound to RNA spin column membrane</li><li>● Ethanol carryover</li></ul>
RNA Degradation	<ul style="list-style-type: none"><li>● Harvested animal tissue not immediately stabilized</li><li>● Inappropriate handling of starting material</li><li>● RNase contamination</li></ul>