

# Total RNA Maxi Kit (Tissue)

*For research use only*

<b>Sample</b>	: 100-200 mg of animal tissue and cultured animal cells (up to $1 \times 10^8$ )
<b>Yield</b>	: 50-300 $\mu$ g
<b>Format</b>	: spin column
<b>Operation time</b>	: within 60 minutes
<b>Elution volume</b>	: 500 $\mu$ l

**Geneaid**



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## Introduction

The Total RNA Maxi Kit (Tissue) was designed specifically for purifying total RNA from a variety of animal tissue and cells. 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 Maxi Kit (Tissue) is tested on a lot-to-lot basis by isolating total RNA from a 100 mg animal tissue sample. The purified RNA is quantified with a spectrophotometer and checked by electrophoresis.

### Kit Contents

Name	RTM02	RTM10	RTM25
RB Buffer	12 ml	60 ml	130 ml
W1 Buffer	10 ml	50 ml	130 ml
Wash Buffer <sup>1</sup> (Add Ethanol)	5 ml (20 ml)	25 ml (100 ml)	50 ml (200 ml)
12.5 ml (50 ml)			
RNase-free water	1 ml	6 ml	30 ml
RB Maxi Column	2 pcs	10 pcs	25 pcs
Filter Column	2 pcs	10 pcs	25 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 the 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 Maxi Kit (Tissue) Protocol

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

### 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<sub>2</sub>, 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<sub>2</sub>, 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 100 mg (up to 200 mg) of fresh or frozen animal tissue. (If using frozen animal tissue, the sample <b>MUST</b> have been flash frozen in liquid nitrogen and immediately stored at -70°C until use, to avoid RNA Degradation).</li> <li>● Grind the sample under liquid nitrogen to a fine powder.</li> <li>● Add <b>5 ml of RB Buffer</b> and 50 <math>\mu</math>l of <math>\beta</math>-mercaptoethanol to the ground sample.</li> <li>● Transfer the mixture to a 15 ml centrifuge tube and mix by vortex.</li> <li>● Incubate at room temperature for 5 minutes.</li> <li>● Place a <b>Filter Column</b> in a 50 ml centrifuge tube. Add the sample mixture to the column.</li> <li>● Centrifuge for 5 minutes at 14-16,000 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 a ½ volume of absolute ethanol to the clarified filtrate from Step 1 and vortex immediately (eg. add 2.5 ml of ethanol to 5 ml of filtrate).</li> <li>● Place a <b>RB Maxi Column</b> in a 50 ml centrifuge tube.</li> <li>● Transfer the ethanol-added mixture to the <b>RB Maxi Column</b>.</li> <li>● Centrifuge at 14-16,000 x g for 5 minutes and discard the flow-through.</li> </ul> <hr/> <p>Optional Step 1: DNA Residue Degradation (see options above)</p>
Step 3 Wash	<ul style="list-style-type: none"> <li>● Add <b>4 ml of W1 Buffer</b> into the <b>RB Maxi Column</b>.</li> <li>● Centrifuge at 14-16,000 x g for 3 minutes.</li> <li>● Discard the flow-through and place the <b>RB Maxi Column</b> back in the 50 ml centrifuge tube.</li> <li>● Add <b>6 ml of Wash Buffer</b> (ethanol added) into the <b>RB Maxi Column</b>. Centrifuge at 14-16,000 x g for 3 minutes.</li> <li>● Discard the flow-through and place the <b>RB Maxi Column</b> back in the 50 ml centrifuge tube.</li> <li>● Add <b>6 ml of Wash Buffer</b> (ethanol added) into the <b>RB Maxi Column</b>. Centrifuge at 14-16,000 x g for 3 minutes.</li> <li>● Discard the flow-through and place the <b>RB Maxi Column</b> back in the 50 ml centrifuge tube.</li> <li>● Centrifuge at 14-16,000 x g for 10 minutes to dry the column matrix.</li> </ul>
Step 4 RNA Elution	<ul style="list-style-type: none"> <li>● Place the dried <b>RB Maxi Column</b> in a clean 50 ml centrifuge tube (RNase-free).</li> <li>● Add <b>500 <math>\mu</math>l of RNase-free water</b> into the center of the column matrix.</li> <li>● Let stand for 5 minutes or until the water is absorbed by the matrix.</li> <li>● Centrifuge at 14-16,000 x g for 5 minutes to elute the purified RNA.</li> </ul> <hr/> <p>Optional Step 2: DNA Residue Degradation (see options above)</p>

## Total RNA Maxi Kit (Tissue) Cultured Cell Protocol

- Additional requirements:  $\beta$ -mercaptoethanol, centrifuge tubes (RNase-free), PBS (phosphate-buffered saline), 0.10–0.25% Trypsin, absolute ethanol

### 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<sub>2</sub>, 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<sub>2</sub>, 50  $\mu$ g/ml BSA at 25°C} to the final elution sample. Let stand for 10 minutes at room temperature.

Step 1 Lysis	<p><b>Suspension cultured animal cells</b></p> <ul style="list-style-type: none"> <li>● Transfer the cells (up to <math>1 \times 10^8</math>) to a 15 ml centrifuge tube and harvest with centrifugation for 5 minutes at 300 x g.</li> <li>● Remove the supernatant.</li> <li>● Add <b>5 ml of RB Buffer</b> and 50 <math>\mu</math>l of <math>\beta</math>-mercaptoethanol to the ground sample and mix by vortex.</li> <li>● Centrifuge for 5 minutes at 14-16,000 x g.</li> <li>● Transfer the supernatant to a new 15 ml centrifuge tube.</li> </ul> <hr/> <p><b>Adherent cultured cells: trypsinize the cells before lysis or lyse cells directly in a culture dish</b></p> <p><i>To trypsinize cells</i></p> <ul style="list-style-type: none"> <li>● Remove the medium, and wash the cells with PBS.</li> <li>● Aspirate the PBS and add 0.10–0.25% Trypsin to the PBS to trypsinize the cells.</li> <li>● Once the cells detach, add the medium and transfer the cells to a 15 ml centrifuge tube.</li> <li>● Pellet the cells as suspension cultured animal cells.</li> </ul> <hr/> <p><i>To lyse cells in a culture dish or flask</i></p> <ul style="list-style-type: none"> <li>● Remove the culture medium. Add <b>5 ml of RB Buffer</b> and 50 <math>\mu</math>l of <math>\beta</math>-mercaptoethanol to the culture dish or flask.</li> <li>● Allow the <b>RB Buffer</b> to cover the dish or flask by shaking. Collect the cell lysate with a rubber policeman.</li> <li>● Transfer the sample lysate to a 15 ml centrifuge tube.</li> <li>● Centrifuge for 5 minutes at 14-16,000 x g.</li> <li>● Transfer the supernatant to a new 15 ml centrifuge tube.</li> </ul>
Step 2 RNA Binding	<ul style="list-style-type: none"> <li>● Add a ½ volume of absolute ethanol to the clarified filtrate from Step 1 and vortex immediately (eg. add 2.5 ml of absolute ethanol to 5 ml of filtrate).</li> <li>● Place a <b>RB Maxi Column</b> in a 50 ml centrifuge tube.</li> <li>● Transfer the ethanol-added mixture to the <b>RB Maxi Column</b>.</li> <li>● Centrifuge at 14-16,000 x g for 5 minutes and discard the flow-through.</li> </ul>
Step 3 Wash	<p>Optional Step 1: DNA Residue Degradation</p> <ul style="list-style-type: none"> <li>● Add <b>4 ml of W1 Buffer</b> into the <b>RB Maxi Column</b>.</li> <li>● Centrifuge at 14-16,000 x g for 3 minutes.</li> <li>● Add <b>6 ml of Wash Buffer</b> (ethanol added) into the <b>RB Maxi Column</b>. Centrifuge at 14-16,000 x g for 3 minutes.</li> <li>● Discard the flow-through and place the <b>RB Maxi Column</b> back in the 50 ml centrifuge tube.</li> <li>● Add <b>6 ml of Wash Buffer</b> (ethanol added) into the <b>RB Maxi Column</b>. Centrifuge at 14-16,000 x g for 3 minutes.</li> <li>● Discard the flow-through and place the <b>RB Maxi Column</b> back in the 50 ml centrifuge tube.</li> <li>● Centrifuge at 14-16,000 x g for 10 minutes to dry the column matrix.</li> </ul>
Step 4 RNA Elution	<ul style="list-style-type: none"> <li>● Place the dried <b>RB Maxi Column</b> in a clean 50 ml centrifuge tube (RNase-free).</li> <li>● Add <b>500 <math>\mu</math>l of RNase-free water</b> into the center of the column matrix.</li> <li>● Let stand for 5 minutes or until the water is absorbed by the matrix.</li> <li>● Centrifuge at 14-16,000 x g for 5 minutes to elute the purified RNA.</li> </ul> <p>Optional Step 2: DNA Residue Degradation (see options above)</p>

## Troubleshooting

Problem	Possible Reasons/Solution
Clogged 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>