

# miRNA Isolation Kit

*For research use only*

**Sample:** up to 100 mg of tissue, up to  $1 \times 10^6$  cultured cells

**RXNS:** 50

**Format:** phenol/chloroform/spin column purification

**Time:** within 30 minutes

**Elution volume:** 50-100  $\mu$ l

**Storage:** dry at room temperature (15-25°C) for up to 9 months

**Geneaid**



CERTIFICATE NO. QAIC/TW/50077

**ISO 9001:2008 QMS**

## Introduction

The miRNA Isolation Kit provides a quick and easy spin column system for purifying and enriching micro RNAs (miRNAs) and other small cellular RNAs from a wide variety of tissue and cells. Since miRNAs are vital for regulating gene expression, this kit is optimized for isolation of small RNA molecules while removing larger RNAs and minimizing genomic DNA contamination for improved sensitive downstream applications.

## Quality Control

The quality of the miRNA Isolation Kit is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system. Purified miRNA is resolved in 50  $\mu$ l of Release Buffer and a 1/10 volume aliquot (5  $\mu$ l) is analyzed by electrophoresis on a 2% agarose gel.

## Kit Contents

Name	RMI004	RMI050
Lysis Buffer	1 ml	12 ml
Mi Buffer	1.5 ml	1.5 ml
Wash Buffer <sup>1</sup> (Add Ethanol)	250 $\mu$ l (1 ml)	12.5 ml (50 ml)
Release Buffer	1 ml	6 ml
RNA Column	8 pcs	100 pcs
2 ml Collection Tube	8 pcs	100 pcs
Micropestle	4 pcs	50 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
Presto™ Mini RNA Bacteria Kit	50/100/300 preps	RBB050/100/300
Presto™ Mini RNA Yeast Kit	50/100/300 preps	RBY050/100/300
miRNA Isolation Kit	50 preps	RMI050
96-Well Total RNA Kit	2/4/10 x 96 Wells	RBP02/04/10

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

## Caution

Buffers contain harmful irritants. During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.

## Steps to prevent RNase contamination

Disposable and nondisposable plasticware and automatic pipettes should be sterile and used only for RNA procedures.

## References

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

## miRNA Isolation Kit Protocol

- Additional Requirements: Trypsin, phosphate-buffered saline (PBS), ddH<sub>2</sub>O saturated phenol, chloroform, absolute ethanol, microcentrifuge tubes, pipette tips, (optional) RNase-free water

Sample Preparation	<p><b>Tissue</b></p> <ul style="list-style-type: none"> <li>• Remove frozen tissue samples from storage or excise fresh tissue samples.</li> <li>• Transfer up to 100 mg of fresh or frozen tissue to a 1.5 ml microcentrifuge tube then proceed to Step 1 Lysis. NOTE: Ensure frozen tissue does not thaw prior to adding Lysis Buffer.</li> </ul> <p><b>Adherent Cultured Animal Cells</b></p> <ul style="list-style-type: none"> <li>• Remove the culture medium and wash cells in PBS.</li> <li>• Aspirate PBS then add 0.10-0.25% Trypsin in PBS.</li> <li>• Once cells have detached, add the medium and transfer to a 15 ml centrifuge tube.</li> <li>• Proceed with Suspension Cultured Animal Cells.</li> </ul> <p><b>Suspension Cultured Animal Cells</b></p> <ul style="list-style-type: none"> <li>• Transfer cells (up to <math>1 \times 10^6</math>) to a 1.5 ml microcentrifuge tube.</li> <li>• Harvest by centrifugation for 5 minutes at 300 x g.</li> <li>• Carefully remove the supernatant completely by aspiration then proceed to Step 1 Lysis.</li> </ul>
Step 1 Lysis	<p><b>Tissue:</b> Add <b>200 µl of Lysis Buffer</b> then use a <b>Micropestle</b> to grind the tissue until it is dissolved completely.</p> <p><b>Cultured cell pellet:</b> Add <b>200 µl of Lysis Buffer</b> then vortex vigorously until the pellet is dissolved completely.</p> <ul style="list-style-type: none"> <li>• Incubate at room temperature for 10 minutes.</li> </ul> <p>At this time, pre-heat the required <b>Release Buffer</b> (50 µl/sample) to 65°C (for Step 5 Elution).</p>
Step 2 RNA Precipitation	<ul style="list-style-type: none"> <li>• Add <b>20 µl of Mi Buffer</b>.</li> <li>• Add <b>180 µl of ddH<sub>2</sub>O saturated phenol and 40 µl of chloroform</b>.</li> <li>• Vortex vigorously for 2 minutes then centrifuge at 14-16,000 x g for 3 minutes.</li> <li>• Transfer the upper phase to a clean 1.5 ml microcentrifuge tube.</li> <li>• Add a <b>35% volume of absolute ethanol to the upper phase</b> and mix well by shaking vigorously. If the upper phase volume is 200 µl, 108 µl of absolute ethanol should be added, e.g. <math>108/(200+108)=0.35</math>.</li> </ul>
Step 3 RNA Binding	<ul style="list-style-type: none"> <li>• Place a <b>RNA Column</b> in a <b>2 ml Collection Tube</b> and transfer the ethanol-added mixture to the <b>RNA Column</b>.</li> <li>• Incubate for 1 minute at room temperature then centrifuge at 14-16,000 x g for 30 seconds.</li> <li>• Transfer the filtrate to a new 1.5 ml microcentrifuge tube.</li> <li>• Add a <b>70% volume of absolute ethanol to the filtrate</b> and mix well by shaking vigorously. If the filtrate volume is 290 µl, 676 µl of absolute ethanol should be added, e.g. <math>676/(290+676)=0.70</math>.</li> <li>• Place a new <b>RNA Column</b> in a <b>2 ml Collection Tube</b> then transfer the mixture to the <b>RNA Column</b>.</li> <li>• Incubate for 1 minute at room temperature.</li> <li>• Centrifuge at 14-16,000 x g for 30 seconds to allow the miRNA to bind to the <b>RNA Column</b> membrane.</li> </ul>
Step 4 Wash	<ul style="list-style-type: none"> <li>• Add <b>200 µl of Wash Buffer</b> (make sure ethanol was added) to the <b>RNA Column</b>.</li> <li>• Incubate for 1 minute at room temperature.</li> <li>• Centrifuge at 14-16,000 x g for 1 minute to completely remove the liquid residue.</li> <li>• Place the <b>RNA Column</b> in a clean 1.5 ml microcentrifuge tube.</li> </ul>
Step 5 Elution	<ul style="list-style-type: none"> <li>• Add <b>50 µl of Release Buffer</b> (pre-heated to 65°C) into the CENTER of the <b>RNA Column</b>.</li> <li>• Incubate for 3 minutes at room temperature.</li> <li>• Centrifuge at 14-16,000 x g for 3 minutes to recover the miRNA.</li> </ul> <p>The purified miRNA can be further concentrated using a standard ethanol precipitation procedure then redissolved in a small volume of RNase-free water.</p>
QC Analysis	<p>Use a 1/5 volume to run on a polyacrylamide gel to check the quality. The majority of RNA visible on the gel should be &lt;100 nt in size, with the major bands corresponding to tRNAs. The 5S and 5.8S rRNA species may also be visible. These tRNA and small rRNA bands should be clear and distinct. miRNA (21-22 nt) are typically not visible on the gel image.</p>

## Troubleshooting

Problem	Possible Reasons/Solution
Clogged Column	<ul style="list-style-type: none"> <li>• Insufficient disruption and/or homogenization</li> <li>• Too much starting material</li> <li>• Centrifugation temperature was too low (should be 20°C to 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 the RB Column membrane</li> <li>• Ethanol carryover</li> </ul>
RNA Degradation	<ul style="list-style-type: none"> <li>• Harvested sample not immediately stabilized</li> <li>• Inappropriate handling of starting material</li> <li>• RNase contamination</li> </ul>