

# miRNA Isolation Kit

*For research use only*

<b>Sample</b>	: 100 mg of tissue or cultured cells (up to $1 \times 10^6$ ) and 200 $\mu$ l of fresh blood
<b>Reactions</b>	: 100
<b>Storage</b>	: room temperature
<b>Operation time</b>	: 30 minutes
<b>Format</b>	: spin column



Revised: 10/21/09

## Introduction

The miRNA Isolation Kit was designed for purifying micro RNAs (miRNAs) and other small cellular RNAs from tissue samples, cultured cells or fresh blood. Purification of miRNAs allows for research into biologically significant pathways for gene regulation. Standard protocols for isolating total RNA and mRNA are not optimized for isolation of small RNA molecules and result in the loss of substantial amounts of miRNAs and other small RNA. In addition, removal of the predominantly larger RNAs is required for accurate analysis of miRNA expression by qPCR or microarray analysis. This kit ensures purification of small RNA with minimal contamination from large RNA molecules or genomic DNA. In the presence of chaotropic salt, and various ethanol concentrations in the solvent, RNA molecules (of various sizes) are selectively bound by the glass fiber matrix (1).

## Kit Contents

Name	IB47370	IB47371
Lysis Buffer	1 ml	25 ml
Mi Buffer	1.5 ml	3 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	200 pcs
2 ml Collection Tube	8 pcs	200 pcs

## Order Information

Product Name	Package size	Cat. No.
Total RNA Mini Kit (Tissue)	50/100 preps	IB47301/02
Total RNA Maxi Kit (Tissue)	10 preps	IB47310
Total RNA Mini Kit (Blood/Cultured Cell)	50/100/300 preps	IB47321/22/23
Total RNA Maxi Kit (Blood/Cultured Cell)	10 preps	IB47330
Total RNA Mini Kit (Plant)	50/100 preps	IB47341/342
Total RNA Maxi Kit (Plant)	10 preps	IB47350
96-Well Total RNA Kit	4/10 x 96 Wells	IB47360/361
miRNA Isolation Kit	100 preps	IB47371
IBI Isolate	100/500 ml	IB47601/602
Vacuum Manifold (Accessories)	1 SET	IB47500

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

## Caution

Buffers contain harmful irritants. During operation, always wear a lab coat, disposable gloves, and protective goggles.

## Additional requirements

absolute ethanol, ddH<sub>2</sub>O saturated phenol, chloroform, microcentrifuge tubes

## References

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

## miRNA Isolation Kit Protocol

Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).

RBC Lysis (for blood only)	<ul style="list-style-type: none"><li>● Add 3 x RBC lysis buffer to the fresh blood sample (eg. Add 600 µl of RBC lysis buffer to 200 µl of fresh blood).</li><li>● Let stand at room temperature for 5 minutes.</li><li>● Centrifuge at 2,000 x g for 5 minutes.</li><li>● Discard the supernatant completely and proceed with Step 1 Lysis.</li></ul>
Step 1 Lysis	<ul style="list-style-type: none"><li>● Add <b>200 µl of Lysis Buffer</b> into a 1.5 ml microcentrifuge tube containing 100 mg of tissue or cultured cell pellet (up to <math>1 \times 10^6</math>) or blood cells.</li><li>● Vortex tissue and cultured cell samples vigorously until the pellet is dissolved completely. For blood cells, pipette repeatedly until the pellet is dissolved completely.</li><li>● Incubate at room temperature for 10 minutes.</li></ul>
Step 2 RNA Precipitation	<ul style="list-style-type: none"><li>● Add <b>20 µl of Mi Buffer</b> to the 1.5 ml microcentrifuge tube.</li><li>● Add 180 µl of ddH<sub>2</sub>O saturated phenol and 40 µl of chloroform into the 1.5 ml microcentrifuge tube.</li><li>● Vortex vigorously for 2 minutes.</li><li>● Centrifuge at 12,000 rpm for 3 minutes.</li><li>● Transfer the upper phase to a clean 1.5 ml microcentrifuge tube.</li><li>● Add a 0.35 volume of absolute ethanol to the upper phase and mix well by shaking (eg. add 108 µl of ethanol to 200 µl of upper phase).</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.</li><li>● Centrifuge at 12,000 rpm for 30 seconds.</li><li>● Transfer the filtrate to a new 1.5 ml microcentrifuge tube (RNase free).</li><li>● Add a 0.70 volume of absolute ethanol to the filtrate and mix well by shaking (eg. add 676 µl of ethanol to 290 µl of filtrate).</li><li>● Place a new <b>RNA Column</b> in a <b>2 ml Collection Tube</b> and transfer the mixture to the <b>RNA Column</b>.</li><li>● Incubate for 1 minute.</li><li>● Centrifuge at 12,000 rpm 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> (ethanol added) to the <b>RNA Column</b> and incubate for 1 minute.</li><li>● Centrifuge at 12,000 rpm for 1 minute to completely remove the residue liquid.</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> (preheated to 65°C) into the center of the <b>RNA Column</b> and incubate for 3 minutes.</li><li>● Centrifuge at 12,000 rpm for 3 minutes to recover the miRNA. {Note: The purified miRNA can be further concentrated by a standard ethanol precipitation procedure and then re-dissolved in a small volume of ddH<sub>2</sub>O or TE (pH 8.0)}</li></ul>
Step 6 Sample-QC Analysis	<ul style="list-style-type: none"><li>● Use a 1/5 volume to run on a mini agarose gel (or more accurately, a polyacrylamide gel) to check its 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.</li></ul>