MiPure Cell/Tissue miRNA Kit (Spin Column)

RC201-EN

Vazyme

Instruction for Use

Version 23.1

Contents

01/Product Description

MiPure Cell/Tissue miRNA Kit (Spin Column) is suitable for the extraction of small RNAs (miRNA, <200 nt) from fewer than 5 × 10^6 eukaryotic cells or less than 100 mg of animal tissues. The obtained miRNAs are of high purity without large RNAs or genomic DNA contamination, can be directly used for Microarrarys, Northern hybridization, RT-PCR and miRNA library preparation, etc. Based on a combination of high performance RNA Isolater extraction reagent and silica gel column purification technology, the extraction of miRNAs can be completed within 1 h with this kit. This kit can also be used to isolate and extract large RNAs (>200 nt) or total RNA (including miRNAs) to meet different needs.

02/Components

	Components	RC201-EN (50 rxns)
BOX 1	RNA Isolater	60 ml
BOX 2	Buffer miRW1	30 ml
	Buffer miRW2	20 ml
	RNase-free ddH_2O	30 ml
	MiPure RNAspin Column	50
	MiPure miRNA Column	50
	2 ml Collection Tube	100

03/Storage

Store BOX 1 at 2 ~ 8℃ and protect from light. Adjust the shipping method according to the destination.

Store BOX 2 at 15 ~ 25℃ and transport at room temperature.

04/Applications

Animal tissues (10 - 100 mg)

Cells (1 × 10^2 - 5 × 10^6)

05/Self-prepared Materials

Absolute ethanol; trichloromethane (chloroform); 80% ethanol freshly prepared with RNase-free ddH_2O (500 µl is required for each sample); 1.5 ml RNase-free centrifuge tubes; RNase-free tips; microcentrifuge; low-temperature centrifuge; suitable homogenization tools;

Before using it for the first time, add absolute ethanol to Buffer miRW1 and Buffer miRW2 according to the table below, and store at room temperature.

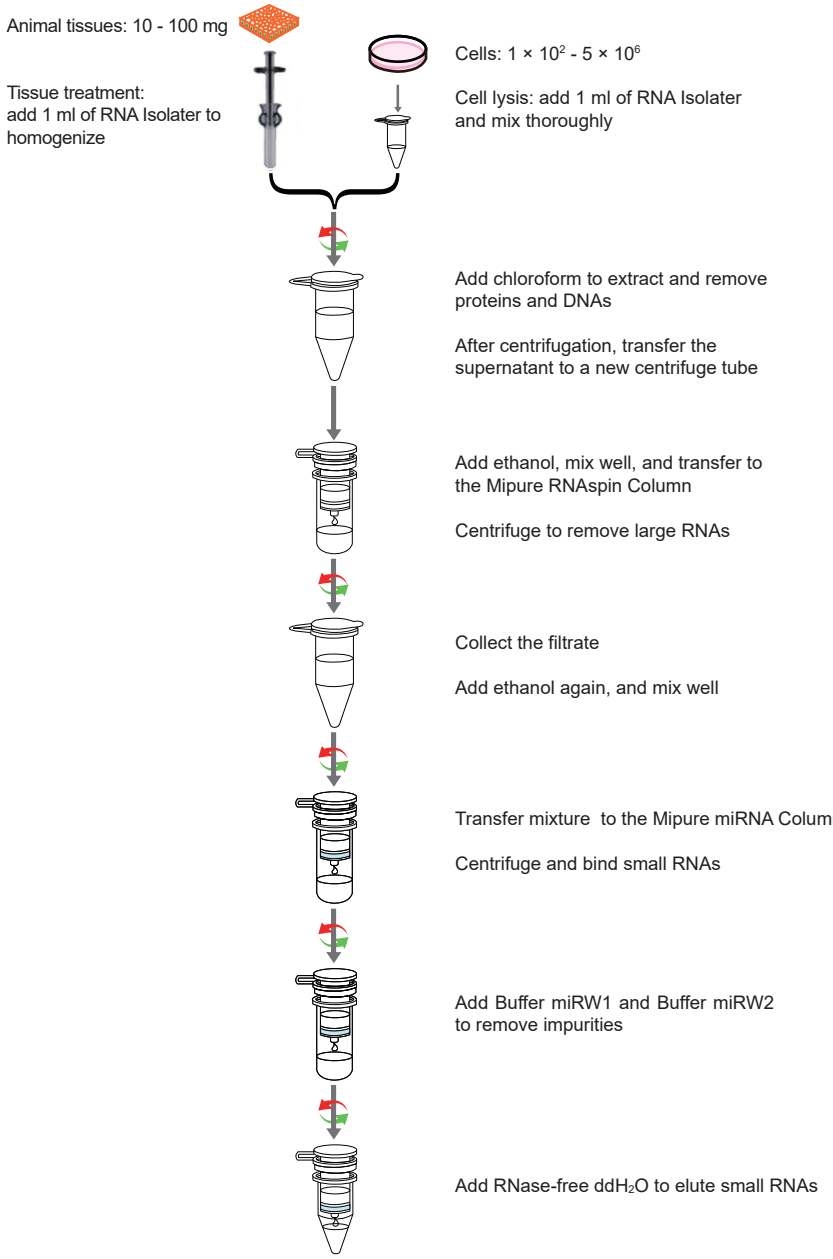
Reagent Name	Buffer miRW1	Buffer miRW2
Amount of absolute ethanol to be added (ml)	60	80

06/Notes

For research use only. Not for use in diagnostic procedures.

1. The RNA Isolater of this product contains phenol, which is toxic and corrosive. If it was inhaled, touched or swallowed, etc., it will cause poisoning, burns, and other bodily harm. When using this product, protective items should be worn, such as suitable lab coat, disposable gloves, protective goggles, masks, etc.
2. Unless otherwise specified, all operating procedures shall be carried out at room temperature (15 ~ 25℃).
3. The RNA yield and quality are related to the amount of tissue samples and elution volume, so it is recommended to lyse 10 - 100 mg of tissues or no more than 5×10^6 cells per 1 ml of RNA Isolater. The elution volume should be no less than 30 μl. Otherwise, the RNA yield will be affected.
4. When using this kit, please wear the lab coat, disposable gloves and masks, etc. to minimize the possibility of RNase contamination.
5. The selection and storage of samples will greatly affect the yield and quality of RNA. It is best to use fresh animal tissues or cells for RNA extraction. If the newly-collected samples are not subjected to extraction immediately, quickly freeze them in liquid nitrogen and store at -70℃ immediately. Repeated freezing and thawing should be avoided. Alternatively, the samples can be homogenized in RNA Isolater immediately, and then store at -70℃. Sample collection and storage should be carried out as quickly as possible to prevent RNA degradation.
6. Samples must be thoroughly disrupted; cell membrane and organelles must be completely disrupted to release RNA as inadequate disruption will affect RNA yield. The homogenization process should be kept at a low temperature to prevent RNA degradation due to heat generated during this process.
7. RNase contamination can be prevented by RNA Isolater, but post-lysis treatment should be performed in RNase-free environment.
8. This kit can remove the genomic DNA in the system, and the purified RNA can usually be used in downstream experiments without DNase treatment. If the downstream experiment is very sensitive to trace amounts of DNA, commercial RNase-free DNase can be used to completely remove it.

07/Mechanism & Workflow



08/Experiment Process

Experiment Preparation

Preventing RNase contamination is critical for RNA extraction. RNase is commonly present in the environment and is very stable. Trace amounts of RNase can quickly degrade RNA. Therefore, protective measures must be taken as follows:

1. Wear disposable gloves; operate in a separate clean area; wear a mask and avoid talking during the operating process. The above measures can effectively prevent contamination from RNase in human sweat and saliva.
2. Use RNase-free laboratory equipments, including tips and centrifuge tubes. All equipments used for RNA experiments should be dedicated and should not be used for other experiments.
3. All reagents used for RNA experiments should be dedicated to prevent cross-contamination. It is recommended to store RNase-free ddH_2O after aliquoting.

Sample Treatment

Homogenization of samples is necessary for RNA extraction. By pipetting or homogenizing, the cells or tissues are quickly dispersed, and the cell wall and cytoplasmic membrane are ruptured, so that the nucleic acids are released into the lysis solution. Inadequate homogenization may result in decreased RNA yield and purity. Several common sample homogenization methods are listed as follows:

1. Liquid nitrogen treatment

   Section an appropriate amount of tissues, and place them in a pre-cooled mortar. Quickly add liquid nitrogen, grind the tissues into powder and pour the powder into a pre-cooled centrifuge tube (note: cool the centrifuge tube in advance to prevent liquid nitrogen from boiling and causing sample loss). After liquid nitrogen has completely evaporated, add an appropriate amount of RNA Isolater and vortex to mix well. As liquid nitrogen grinding can only disrupt the sample, homogenization must be performed to reduce the viscosity of the lysis buffer.
2. Homogenization with a mechanical homogenizer

   Mechanical homogenizers are able to homogenize most tissues and cells in a highly effective manner, disrupting and homogenizing at the same time. Place the sample in a 1 - 5 ml glass tube or centrifuge tube and add RNA Isolater. Insert the rotor into the RNA Isolater and homogenize intermittently at a low temperature and a high speed for 10 - 30 sec each time, keeping the interval the same until the sample is completely homogenized. When using a mechanical homogenizer, the ideal homogenization effect can be achieved within 1 min for general tissues. Most mechanical homogenizers come with rotors of varying sizes, and smaller rotors are suitable for a small volume of lysis buffer.
3. Glass homogenizer

   Transfer the sample and an appropriate amount of lysis buffer into a glass homogenizer, and grind up and down until the tissue is completely disrupted.

08-1/Protocol 1. Extraction of Small RNAs from Cells

This protocol is suitable for small RNAs (<200 nt) enrichment from samples with less than 5×10^6 cultured cells. If large RNAs (>200 nt) do not need to be removed, total RNA (including large RNAs and small RNAs) may be extracted according to Protocol 4 after Step 5.

1. Cell collection
   a. Suspension cells: determine the number of cells. Pellet the appropriate number of cells by centrifuging at 1,800 rpm (300 × g) for 5 min. Carefully discard the culture medium, and proceed to Step 2.
   b. Adherent cells: adherent cells can be either directly lysed in the culture flask/dish or trypsinized and collected as a cell pellet prior to lysis.
   ▲ Direct lysis: determine the number of cells. Completely discard the culture medium, and proceed to Step 2.
   ▲ Trypsin digestion treatment: determine the number of cells. Discard the culture medium and add an appropriate amount of PBS to wash the cells. Discard the PBS, and add PBS containing 0.1% - 0.25% of trypsin to digest the cells. After the cells detach from the flask or dish, add culture medium containing serum, transfer to a centrifuge tube, and centrifuge at 1,800 rpm (300 × g) for 5 min. Collect the cells and proceed to Step 2.
2. Add 1 ml of RNA Isolater into the cell sample. Vortex or pipette to treat the cell pellet.
   ▲ For pelleted cells: loosen the cell pellet thoroughly by flicking the tube, then add 1 ml of RNA Isolater, and pipette 10 - 15 times to thoroughly disrupt the cells.
   For direct lysis of adherent cells: after discarding all the culture medium, add 1 ml of RNA Isolater into the culture flask or culture dish. Pipette to completely detach the adherent cells, collect the lysis buffer and transfer to a centrifuge tube.
3. Place the tube containing the homogenate at room temperature for 2 - 3 min to allow sufficient lysis of the cells.
   ▲ The sample from this step can be kept for one week at 2 ~ 8℃ and for more than six months at -70 ~ -20℃.
4. Add 200 µl of chloroform to the lysis buffer. Shake the tube vigorously for 15 sec and place the tube at room temperature for 3 min.
   ▲ Vortex instead of oscillation may lead to genomic DNA contamination.
5. Centrifuge at 4℃, 12,000 rpm (13,400 × g) for 15 min. Transfer 500 µl of supernatant into a new 1.5 ml RNase-free centrifuge tube. Carry out Steps 6 - 16 for small RNAs enrichment, or extract total RNA (including small RNAs and large RNAs) according to Protocol 4.
   ▲ Please carefully transfer the supernatant aqueous phase and avoid pipetting the middle layer and bottom organic phase, so that the subsequent extraction results will not be affected.
6. Add 160 µl of absolute ethanol to the supernatant, and vortex for 10 sec to mix well.
   (**Perform the following centrifugation at room temperature.**)
7. Place the MiPure RNAspin Column in the 2 ml Collection Tube. Transfer the above mixture into the MiPure RNAspin Column. Centrifuge at 12,000 rpm (13,400 × g) for 30 sec.
   ▲ If the extraction of large RNAs is required, retain the MiPure RNAspin Column, and extract large RNAs (>200 nt) according to Protocol 3.
8. Add 0.9 × volume of absolute ethanol to the filtrate, and pipetting up and down 3 - 5 times.
   ▲ For example: if the filtrate volume is 640 µl, 576 µl of absolute ethanol must be added.
9. Place the MiPure miRNA Column into the 2 ml Collection Tube. Transfer half the volume of the mixture into the MiPure miRNA Column. Centrifuge at 12,000 rpm (13,400 × g) for 30 sec.

10. Discard the filtrate and place the MiPure miRNA Column back into the 2 ml Collection Tube. Transfer the remaining mixture into the MiPure miRNA Column, and centrifuge at 12,000 rpm (13,400 × g) for 30 sec.
11. Discard the filtrate and place the MiPure miRNA Column back into the 2 ml Collection Tube. Add 500 µl of Buffer miRW1 (**check if absolute ethanol has been added in advance!**) to the MiPure miRNA Column. Incubate at room temperature for 1 min, and centrifuge at 12,000 rpm (13,400 × g) for 30 sec.
12. Discard the filtrate and place the MiPure miRNA Column back into the 2 ml Collection Tube. Add 500 µl of Buffer miRW2 (**check if absolute ethanol has been added in advance!**) to the MiPure miRNA Column. Incubate at room temperature for 1 min, and centrifuge at 12,000 rpm (13,400 × g) for 30 sec.
13. Discard the filtrate and place the MiPure miRNA Column back into the 2 ml Collection Tube. Add 500 µl of 80% ethanol (**must be freshly prepared with RNase-free ddH_2O**) into the MiPure miRNA Column. Incubate at room temperature for 1 min, and centrifuge at 12,000 rpm (13,400 × g) for 30 sec.
14. Discard the filtrate and place the MiPure miRNA Column back into the 2 ml Collection Tube. Centrifuge the empty column at 12,000 rpm (13,400 × g) for 2 min to dry the MiPure miRNA Column membrane. This step can thoroughly remove the residual ethanol in the MiPure miRNA Column.
15. Transfer the MiPure miRNA Column to a new 1.5 ml RNase-free centrifuge tube. Dry at room temperature for 2 - 5 min, add 30 - 50 µl of RNase-free ddH_2O to the center of the MiPure miRNA Column membrane. Incubate at room temperature for 2 min. Centrifuge at 12,000 rpm (13,400 × g) for 1 min to collect the filtrate.
    ▲ The minimum elution volume of the MiPure miRNA Column is 30 µl, and less elution volume will cause a reduction in RNA elution efficiency.
16. Discard the MiPure miRNA Column, and store the miRNA at -70℃.

08-2/Protocol 2. Extraction of Small RNAs from Animal Tissues

This protocol is suitable for small RNAs (<200 nt) enrichment from less than 100 mg of animal tissues. If large RNAs (>200 nt) do not need to be removed, total RNA (including large RNAs and small RNAs) can be extracted according to Protocol 4 after Step 6.

1. Amount of tissues

    The amount of tissues is critical for RNA yield and purity. The amount of tissues used for the kit can be as low as 0.01 mg, but the maximum amount of tissues used depends on the RNA, protein and impurity content in the samples.

    ▲ The RNA content of animal brain tissues and adipose tissues are relatively low, thus the amount of tissues used can be up to 100 mg.

    ▲ Animal liver, spleen, kidney, and thymus, etc. are rich in RNA, thus the amount of tissues used should not exceed 20 mg.

    ▲ Heart, muscle, and skin have medium abundance of RNA, thus the amount of tissues used should not exceed 50 mg.

    ▲ The binding capability of the MiPure miRNA Column is 200 µg. An excessive amount of tissues will cause RNA degradation and large RNAs contamination. If there is no relevant information, it is recommended that the starting amount can be 30 mg, and the amount of tissues used may be increased or reduced based on the results obtained.

2. Lysis and homogenization of tissues: for 10 - 100 mg of tissues, add 1 ml of RNA Isolater.
   ▲ Select an appropriate homogenization method. Refer to “Sample Treatment” on Page 5 for details.
3. Incubate at room temperature for 2 - 3 min for complete lysis of the tissues.
4. (Optional) Centrifuge at 4℃, 12,000 rpm (13,400 × g) for 10 min. Carefully transfer the supernatant into a new centrifuge tube.
   ▲ For adipose samples, a layer of grease will float on the surface of the solution after centrifugation. Carefully transfer the clear liquid below into a new tube.
5. Add 200 µl of chloroform to the lysis buffer or supernatant. Shake the tube vigorously for 15 sec and place the tube at room temperature for 3 min.
   ▲Vortex instead of oscillation may introduce genomic DNA contamination.
6. Centrifuge at 4℃, 12,000 rpm (13,400 × g) for 15 min. Transfer 500 µl of supernatant into a new 1.5 ml RNase-free centrifuge tube.
   ▲ Please carefully pipette the supernatant aqueous phase and avoid pipetting the middle layer and bottom organic phase, so that the subsequent extraction results will not be affected.

Carry out Steps 6 - 16 for small RNAs enrichment according to Protocol 1, or extract total RNA (including small RNAs and large RNAs) according to Protocol 4.

08-3/Protocol 3. Extraction of Large RNAs

This protocol is suitable for the extraction of large RNAs from all types of samples.

1. Take the MiPure RNAspin Column in Protocol 1 or 2 and place in a 2 ml Collection Tube.
2. Add 500 µl of Buffer miRW1 (**check if absolute ethanol has been added in advance!**) to the column, incubate at room temperature for 1 min, and centrifuge at 12,000 rpm (13,400 × g) for 30 sec.
3. Discard the filtrate and place the MiPure RNAspin Column back into the 2 ml Collection Tube. Add 500 µl of Buffer miRW2 (**check if absolute ethanol has been added in advance!**) to the column. Incubate at room temperature for 1 min, and centrifuge at 12,000 rpm (13,400 × g) for 30 sec.
4. Discard the filtrate and place the MiPure RNAspin Column back into the 2 ml Collection Tube. Add 500 µl of 80% ethanol (must be freshly prepared with RNase-free ddH_2O) to the column, incubate at room temperature for 1 min, and centrifuge at 12,000 rpm (13,400 × g) for 30 sec.
5. Discard the filtrate and place the MiPure RNAspin Column back into the 2 ml Collection Tube. Centrifuge the empty column at 12,000 rpm (13,400 × g) for 2 min to dry the MiPure RNAspin Column memebrane.
6. Transfer the MiPure RNAspin Column to a 1.5 ml RNase-free centrifuge tube. Dry at room temperature for 2 - 5 min, add 30 - 50 µl of RNase-free ddH_2O to the center of the MiPure RNAspin Column membrane, and incubate at room temperature for 2 min. Centrifuge at 12,000 rpm (13,400 × g) for 1 min.
7. (Optional) Add another 30 - 50 µl of RNase-free ddH_2O to the center of the MiPure RNAspin Column membrane, and incubate at room temperature for 2 min. Centrifuge at 12,000 rpm (13,400 × g) for 1 min.
   ▲ The minimum elution volume of the MiPure RNAspin Column is 30 µl, and less elution volume will cause a reduction in RNA elution efficiency. If the yield of RNA exceeds 30 µg, it is recommended to perform a second elution according to Step 7 to obtain an even higher yield.
8. Discard the MiPure RNAspin Column, store the large RNAs at -70℃.

08-4/Protocol 4. Extraction of total RNA (Including Large and Small RNAs)

This protocol is suitable for the direct extraction of total RNA, including large RNAs and small RNAs from cells and tissues. In qRT-PCR quantitative analysis experiments, the C_T value of miRNA obtained through this method is more stable. However, the total yield is slightly lower than only extracting small RNAs.

1. Transfer 500 µl of the supernatant (supernatant after chloroform extraction) in Protocol 1 and 2 into a new centrifuge tube.
   ▲ Please carefully transfer the supernatant aqueous phase without disturbing the middle layer and bottom organic phase, so that the subsequent extraction results will not be affected.
2. Add 750 µl of absolute ethanol to the supernatant. Vortex for 10 sec to mix well.
3. Place the MiPure RNAspin Column into a 2 ml Collection Tube. Transfer half the volume of the mixture into the column, and centrifuge at 12,000 rpm (13,400 × g) for 30 sec.
4. Discard the filtrate and place the MiPure RNAspin Column back into the 2 ml Collection Tube. Transfer the remaining mixture into the MiPure RNAspin Column, and centrifuge at 12,000 rpm (13,400 × g) for 30 sec.
5. Discard the filtrate and place the MiPure RNAspin Column back into the 2 ml Collection Tube. Add 500 µl of Buffer miRW1 (**check if absolute ethanol has been added in advance!**) to the column. Incubate at room temperature for 1 min, and centrifuge at 12,000 rpm (13,400 × g) for 30 sec.
6. Discard the filtrate and place the MiPure RNAspin Column back into the 2 ml Collection Tube. Add 500 µl of Buffer miRW2 (**check if absolute ethanol has been added in advance!**) to the column. Incubate at room temperature for 1 min, and centrifuge at 12,000 rpm (13,400 × g) for 30 sec.
7. Discard the filtrate and place the MiPure RNAspin Column back into the 2 ml Collection Tube. Add 500 µl of 80% ethanol (**must be freshly prepared with RNase-free ddH_2O**) into the column. Incubate at room temperature for 1 min, and centrifuge at 12,000 rpm (13,400 × g) for 30 sec.
8. Discard the filtrate and place the MiPure RNAspin Column back into the 2 ml Collection Tube. Centrifuge the empty column at 12,000 rpm (13,400 × g) for 2 min to dry the MiPure RNAspin Column membrane.
9. Transfer the MiPure RNAspin Column to a 1.5 ml RNase-free centrifuge tube. Add 30 - 50 µl of RNase-free ddH_2O to the center of the MiPure RNAspin Column membrane. Incubate at room temperature for 2 min. Centrifuge at 12,000 rpm (13,400 × g) for 1 min.
10. (Optional) Add another 30 - 50 µl of RNase-free ddH_2O to the center of the MiPure RNAspin Column membrane. Incubate at room temperature for 2 min. Centrifuge at 12,000 rpm (13,400 × g) for 1 min.
    ▲ The minimum elution volume of the MiPure RNAspin Column is 30 µl, and less elution volume will cause a reduction in RNA elution efficiency. If the yield of RNA exceeds 30 µg, it is recommended to perform a second elution according to Step 10 to obtain an even higher yield.
11. Discard the MiPure RNAspin Column, and store the total RNA (including small RNAs) at -70℃.

09/FAQ & Troubleshooting

FAQ	Reasons	Solutions
The phases are not well separated after centrifugation	Chloroform is not added or chloroform is not pure	Ensure that chloroform is added, and chloroform does not contain isoamyl alcohol or other additives.
	After adding chloroform, the homogenate is not sufficiently mixed	After adding chloroform, the mixture must be vigorously oscillated and mixed well for 15 sec. Inversion or vortex will cause unapparent separation or DNA contamination. If the phases are not well separated after centrifugation, repeat the oscillation and incubation step, then centrifuge again.
	The sample contains organic solvents	If the sample contains organic solvents such as DMSO, ethanol, and strong alkaline reagent. These can interfere with the phase separation.
Low RNA yield	Inadequate homogenization	When treating cultured cells, repeatedly pipette the lysis buffer for lysis; When treating animal tissues, it is recommended to homogenize the sample with a mechanical homogenizer.
	Excessive starting material	Choose the amount of sample according to the reference amount provided in the instruction manual and the actual situation.
	Low RNA elution efficiency	RNase-free ddH_2O is not added to the membrane, or the elution volume is inadequate. 30 - 50 µl of pre-heated RNase-free ddH_2O can be added to the membrane. Incubate at room temperature for 2 min, and centrifuge to elute RNA.
RNA degradation	Excessive amount of tissues/cells used	Reduce the amount of sample used. Using the correct amount of sample is necessary to obtain the desired result.
	RNase contamination	Avoid RNase contamination in the operating process. Refer to “Experiment Preparation” on Page 5.
Downstream experiment results are not ideal	Salt contamination	Add Buffer miRW2 or 80% ethanol, incubate for 2 min and then centrifuge.
	Ethanol contamination	Centrifuge the empty column at a rotational speed higher or equal to 12,000 rpm (13,400 × g) for 2 min.
	Detachment of membrane	Silica gel membrane detached into the products is insoluble; through centrifugation at 12,000 rpm (13,400 × g) for 2 min, only the liquid components containing small RNAs.

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