Note: *for laboratory research use only*

microRNA Fast Extraction Kit
(Spin-column)

Cat. # RP5301 (50 preps)

Signalway Biotechnology

I. Kit Content, Storage, and Stability

Content	Storage	50 preps (RP5301)
Buffer MRL	4℃	55 ml
Buffer RW	RT	15 ml
		Add 60ml ethanol before use.
RNase-free H_2O	RT	10 ml
Buffer RH	RT	9 ml RNase-free H_2O
		Add 21ml ethanol before use.
RNase-free Spin-column RA	RT	50
RNase-free Spin-column RB	RT	50
Collection Tube (2ml)	RT	50

All reagents will be stable for 12 months if stored properly.

Reminders:

1 · ***Please add ethanol to buffer WB and Buffer RH before use; mix adequately and then check the box on the label showing ethanol was added!***

2 · Buffer may precipitate under low temperature. Incubate the buffer at 37°C for a few minutes **until buffer is clear**, and then let the buffer cool to RT before use.

3 · Buffer MRL can be transported at RT and should be stored at **4℃ in the dark.**

4 · ***Proteinase K is provided in freeze-dried powder for activity and transportation. Centrifuge a few seconds and add 1 ml sterile water to the tube.*** Because multiple freeze-thaws may affect enzyme activity, store aliquots under -20℃.

5 · Keep the reagent lids tightly caped when not in use to prevent evaporation, oxidation, and changes in pH.

II. Principle

The kit applies improved guanidine thiocyanate-phenol one-step method to lyse samples and inactivate ribonucleases. Genomic DNA and 18s and 28s RNA are removed by the spin-column RA. Then microRNA (including miRNA, snRNA, and other RNA less than 200bp) is absorbed by the spin-column RB. After a serial of elution-centrifugation steps to remove cellular metabolite and proteins, finally microRNA will be eluted from silica membrane by using low salt RNase-free water.

III. Features

1 · It is suitable for miRNA, siRNA, shRNA, and snRNA analysis.

2 · Rapid and convenient. The kit does not contain poisonous phenol and does not need a step of ethanol precipitation.

3 · Small RNAs shorter than 200nt are extracted.

4 · Multi-elution can ensure high-purified microRNA, which can be applied to all kinds of molecular biology experiments. Excellent microRNA yield.

5 · High-purity; unique membrane absorption and specialized washing for removing protein and other debris.

IV.Notes

Please read this section before your experiment.

1 · **To prevent RNA degradation, all the centrifugation steps should be performed under 4℃ unless being specially noted**. Recommend using traditional centrifuge (with up to 13,000 rpm), such as Eppendorf 5415C.

2 · Reagent and buffer may contain a corrosive compound; please wear latex gloves to avoid direct contact with skin, eyes, and clothes**. If contact occurs, wash with water or physiological saline.**

3 · Due to the prevalence of RNases, wear gloves during the procedure and change them whenever contact by reagents occurs; please follow standard laboratory procedures of "Molecular Clone" rules.

** Wear gloves during entire process. Skin often contains bacteria and molds that can contaminate an RNA preparation and be a source of RNases.*

** Use sterile, disposable plastic-ware, and automatic pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipment. For example, a laboratory that is using RNA probes will likely be using RNase A or T1 to reduce background on filters, and any non-disposable items (such as automatic pipettes) can be rich sources of RNases.*

** Treat non-disposable glassware and non-disposable plastic-ware before use to ensure that they are RNase-free. Bake glassware at 200°C overnight, and thoroughly rinse plastic-ware with 0.1N NaOH with 1mM EDTA followed by RNase-free water.*

4 · The integrity of purified RNA may be determined by denaturing agarose gel electrophoresis (or agarose gel electrophoresis). The ratio of 28S to 18S ribosomal RNA should be approximately 2:1 by ethidium bromide staining. There may be the third band about 0.1-0.3 kb (5S RNA and tRNA) and even fourth or fifth band of RNA purified from some plant tissues. The extracted preRNA, hnRNA, and small RNA will appear as some bands at sizes of 7-15 kb.

5 · Chloroform and isoamylol should be prepared by the user.

6 · The routine method to determine the yield and purity of RNA is spectrophotometry (OD_{260}/OD_{280}). Please dissolute RNA by TE. Water will make OD_{280} higher because of lower ion intensity and PH.

7 · The sample, which was mixed with Buffer MRL and was homogenized (without chloroform), can be stored under –60℃--70℃ for a month even longer.

V. Procedure

Please add proper volume of ethanol to buffer RW and Buffer RH before use.

1 · Homogenization

a. Tissues

Homogenize tissue in an appropriate volume of Buffer MRL (50-100 mg/ml) until no visible tissue; for tissue in liquid nitrogen, grind the tissue into fine powder using a mortar and pestle. The volume of sample should not be more than 1/10 total volume of Buffer MRL.

b. Adherent cells

Add Buffer MRL directly to the culture plate, and completely lyse cell by pipetting. The volume of Buffer MRL is decided by the area of culture plate (about 10 cm^2 per 1ml). Genomic DNA may appear and affect the yield if Buffer MRL is not enough.

c. Suspended cells

Harvesting cells in Buffer MRL (animal, plant, fungus, maximum $5\sim10\times10^6$cells per 1ml; bacteria, maximum 1×10^7 cells per 1 ml) in a micro centrifuge tube by centrifuging.

2 · Mix vigorously and incubate for 5 minutes at 4℃ to lyse ribosomal particle completely.

3 · **(Alternative step*)** Centrifuge mixture at 12,000 rpm for 10 minutes at 4°C. Remove upper, aqueous phase to a clean, sterile, and DEPC-treated eppendorf tube.

** This procedure is needed when the sample is rich in proteins, fats, amylase, and other extracellular substances (e.g., muscle and plant tuber).*

4 · Add 200 μl chloroform, cap the lid tightly and mix vigorously 15 seconds, then incubate for 3 minutes under RT.

5 · Centrifuge mixture at 12,000 rpm for 10 minutes at 4°C. Remove upper, aqueous

phase that RNA is present to a clean, sterile, and DEPC-treated eppendorf tube.

6． Precipitate the aqueous phase by the addition of an equal volume (around 500 μl) of Buffer RH **(please check if ethanol is added),** mix gently. Flocculated precipitate may appear.

7． Put solution from last step and the flocculated precipitate into a Spin-column RA (place the spin-column to collection tube).

8． Centrifuge at 10,000 rpm for 45 seconds and collect flow-through (including microRNA)**,** and check the volume of flow-through **(please be acute).** Add 70% ethanol (2/3 times volume of flow-through) and mix gently, then put this mixed solution into a Spin-column RB, centrifuge at 10,000 rpm for 30 seconds at 4°C (Repeat the step until all solution is loaded), and discard flow-through. At this stage, you have Spin-column RA containing macro RNA (18s and 28s) and Spin-column RB containing microRNA.

If you want to harvest macro RNA (18s and 28s) from Spin-column RA, please proceed Step 9-12 with the spin column RA.

If you want to harvest microRNA from Spin-column RB, please proceed Step 9-12 with the spin column RB.

9． Add 700 μl Buffer RW to the Spin-column **(please check if ethanol is added)**，centrifuge at 12,000 rpm for 60 seconds, and discard flow-through liquid.

10． Add 500 μl Buffer RW, centrifuge at 12,000 rpm for 60 seconds，and discard flow-through liquid.

11． Place spin-column back to collection tube，centrifuge at 12,000 rpm for 2

minutes, and discard supernatant to avoid ethanol inhibition of the down-stream procedures.

12 · Place the Spin-column into a new RNase-free tube, add 60-80 μl RNase-free water (Pre-warmed at 65-70℃), hold for 2 minutes at RT. Centrifuge at 12,000 rpm for 1 minute.

13. Keep microRNA at -20℃ or apply for down-stream reactions.

Ⅵ. Trouble shooting

Problem	Possible Reason	Advices
Low microRNA	Tissue not homogenized thoroughly	For tissue in liquid nitrogen, grind tissue into fine powder, after add buffer RL, then completely lyse cells by pipetting or vortaxing; for fresh tissue or plant tissue, grind tissue in Buffer MRL using a mortar and pestle.
	RNA degraded	Using fresh samples
	low RNA in samples	The amount of RNA varies in tissues and cells. Some “low RNA” samples need larger amount of tissues or cells for homogenization.
	Beyond the binding capacity of silica membrane	Using multiple spin-columns RA for the same sample.
	Ethanol not added to Buffer RW	Add the ethanol before use.
OD260/OD280 <1.6	Dissolute RNA by water, which will make OD280 higher because of lower ion intensity and pH.	Dissolute RNA by TE for spectrophotometry analysis.
	Contaminated by proteins and phenol	Don’t take middle and lower phases in Step 5 and ensure proceeding of Step 8.
Genomic DNA contamination	Initial sample beyond the capacity of Buffer MRL	Selecting appropriate amount of sample.
	Sample contains some chemical solvent (such as ethanol, DMSO etc).	Avoid contamination of sample
	extract middle phases in Step 5.	Don’t take middle phases in Step 5.
Degradation and poor integrity of RNA	non-disposable glassware and plasticware not treated before use.	Treat non-disposable glassware and plasticware before use to ensure that it is RNase-free
	Samples were not properly prepared or stored. RNA may have been degraded during sample preparation.	To avoid RNA degradation within samples, immediately homogenize sample with Buffer MRL or freeze samples immediately in liquid nitrogen

		and store at -70°C if they cannot be immediately processed.
	RNA not stored under –60℃–70℃	Store under –60℃–70℃
	RNA degradation in process.	It is essential to work quickly during sample preparation. Maintain sample lysate at 4°C during preparation.
Down-stream RT-PCR not successful.	Forget to do step11, or when take the spin-column out, touching some flow-through including ethanol carelessly. Ethanol will inhibit RT-PCR.	Ensuring to do step11, take the spin-column out carefully, and then put outside for a few minutes for ethanol evaporation.