Note: for laboratory research use only

Serum/Liquid Sample microRNA Fast Extraction Kit (Spin-column)

Cat. # RP5903 (50 preps)

Signalway Biotechnology

Ⅰ. Kit Content, Storage and Stability

Content	Storage	50 preps（DP3111）
Buffer MRL	4℃ in the dark	55 ml
Buffer RW	RT	15 ml
		Add 60ml ethanol before use.
70% ethanol	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 are stable for 12 months when stored properly.

Reminder:

1. ***Add ethanol to Buffer WB and 70% ethanol before use, mix adequately, and then check the box on the label showing ethanol was added!***
2. Precipitate may occur under low temperature, and it will affect RNA yield. Incubate to 37°C for a few minutes **until buffer is clear**, and then let the buffer cool to RT before use. Buffer MRL can be transported at RT, and then keep it at **4℃ in the dark** upon arrival.
3. Keep all of the reagents 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 18 and 28s RNA are removed by the spin-column RA. MicroRNA (including miRNA, snRNA, and other RNA less than 200 bp) is absorbed by the spin-column RB. After a serial of elution-centrifugation steps to remove cellular metabolite and proteins, microRNA will be eluted from silica membrane by using low salt RNase-free water.

Ⅲ. Features:

1. Rapid and convenient. Does not contain poisonous phenol and does not require for ethanol precipitation.
2. Multi-elution can ensure high-purified microRNA, which can be applied to all kinds of molecular biology experiments and down-stream reactions.
3. High-purity; unique membrane absorption and specialized washing for removing protein and other debris.
4. Excellent microRNA yield.

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), for example Eppendorf 5415C or the similar machine.
2. Reagent and buffer may contain a corrosive compound; 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 longer period of time..

Ⅴ. Procedure

Please add proper volume ethanol to buffer RW and bottle of 70% ethanol before use.

1. Take 250 μl serum (blood or other liquid samples) into one 1.5 ml tube and add 750 μl Buffer MRL. Volume ratio of Buffer MRL and sample should be 3:1. 5~10×10^6 cells should be lysed in 750 μl Buffer MRL.
2. Mix vigorously and incubate for 5 minutes to lyse ribosomal particle completely.
3. **(Alternative step*)** Centrifuge mixture at 12,000 g for 10 minutes at 4°C. Remove upper, aqueous phase to a clean, sterile, DEPC-treated eppendorf tube.

   *When the sample is rich in proteins, fats, amylase, and other extracellular substances (e.g., muscle and plant tuber), it needs this procedure.
4. For every 1 mL Buffer MRL, adds 200 μl chloroform, close the lid tightly, and mix vigorously for 15 seconds, then incubate for 3 minutes under RT.
5. Centrifuge mixture at 12,000 g for 10 minutes at 4°C. Remove upper, aqueous phase where RNA is present to a clean, sterile, DEPC-treated eppendorf tube.
6. Precipitate the aqueous phase by the addition of an equal volume (around 500 μl) of 70% ethanol **(please check if ethanol is added!)** and mix gently. Then, flocculated precipitate may appear.
7. Put solution and the flocculated precipitate from step 6 into a Spin-column RA (place the spin-column into 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 g 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 precede Step 9-12 with the spin column RA.**

*** If you want to harvest microRNA from Spin-column RB, please precede 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 g for 60 seconds, and discard flow-through.
10. Add 500 µl buffer RW, centrifuge at 12,000 g for 60 seconds，and discard flow-through liquid.
11. Place spin-column back to collection tube，centrifuge at 12,000 g for 2 minutes, and discard supernatant to avoid ethanol inhibition of 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 g for 1 minute.
13. Keep microRNA at -20℃ or apply for down-stream reactions.

Ⅶ. Troubleshooting

Problem	Possible Reason	Advices
Low microRNA	Tissue not homogenized thoroughly	For tissue in liquid nitrogen, grind tissue into a fine powder, after add buffer RL, then completely lyse cells by pipetting or vortexing; for fresh tissue or plant tissue, grind tissue in Buffer MRL using a mortar and pestle.
	RNA degraded	Take fresh samples for isolation.
	The sample is poor in RNA	There are different RNA percentage in all kinds of tissues and cells. Some “poor RNA” samples need larger amount of tissues or cells for homogenization.
	Beyond the binding maximum of silica membrane	Using multiple spin-columns RA for the same sample.
	Ethanol not added to Buffer RW	Add the ethanol before use.
OD_{260}/OD_{280} <1.6	Dissolute RNA by water, which will make OD_{280} higher because of lower ion intensity and PH.	Please dissolute RNA by TE for spectrophotometry.
	Contaminated by proteins and phenol	Don’t take middle and lower phases in Step5 and ensure to do Step8.
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 these substances.
	May extract middle phases in Step5.	Don’t take middle phases in Step5.

RNA degradation and integrity not well	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 lysis 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.