Paraffin-embedded Tissue Sections MicroRNA Extraction Kit (Spin-column)

Cat.#: **RP 5311(50 preps)**
RP5312 (100 preps)

Signalway Biotechnology

Ⅰ. Kit Content, Storage Condition and Stability

Content	Storage	50preps （RP5311）	100preps （RP5312）
Buffer PTL	RT	15ml	30ml
Protease K	-20℃	1ml	1mlx2
Buffer MRL	4℃ in the dark	55 ml	55mlx2
Buffer RW	4℃ (one month) -20℃(long term)	15 ml H_2O	25ml H_2O
		Add 60ml ethanol (not supplied)	***Add 100ml ethanol (not supplied)***
RNase-free H_2O	RT	10ml	20ml
RNase-free Spin-column RA	RT	50	100
RNase-free Spin-column RB	RT	50	100
Collection Tube（2ml）	RT	100	200
70% ethanol	RT	9ml RNase-free H_2O	18ml RNase-free H_2O
		Add 21ml ethanol (not supplied)	***Add 42ml ethanol (not supplied)***

All reagents, when stored properly, are stable for 12 months.

Notes:

1. ***Please add proper volume ethanol to the bottles labeled Buffer RW and 70% ethanol before starting. Mix well and mark the check box labeled on the bottles to indicate that the ethanol has been added.***
2. All reagents should be clear. 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. All reagents can be transported under room temperature (15℃－25℃). Buffer MRL can be transported under RT and keep at 4℃ upon arrival. *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℃.
4. Please ensure the bottles tightly capped when not in use, prevent reagents from evaporating, oxidation and pH change.

Ⅱ.Principle

The RNApure procedure represents a well-established technology for RNA purification. This technology combines the selective binding properties of a silica

membrane with the speed of microspin technology. A specialized high-salt buffer system allows up to 100 μg of RNA longer than 200 bases to bind to the RNApure silica membrane. Biological samples are first lysed and homogenized in the presence of a highly denaturing guanidine-thiocyanate–containing buffer, which immediately inactivates RNases to ensure intact RNA. Ethanol is added to provide appropriate binding conditions, and the sample is then applied to an RNApure spin-column, where the total RNA binds to the membrane and contaminants are efficiently removed. High-quality RNA is then eluted in 30–100 μl water.

Ⅲ. Features

- Stability, comparable miRNA yield with high quality absorbing membrane.
- High-purity, specific membrane absorption and washing for removing protein and other debris.

Ⅳ. Notes

1. **To prevent RNA degradation, all the centrifuge steps should be made under 4℃, unless having special notes**. Suggest using up to 13,000 rpm traditional centrifuge, for example Eppendorf 5415C.
2. Buffer MRL contain stimulating compound, please wear latex gloves, avoiding skin, eyes and cloth to be contaminated. **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 in 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 nondisposable items (such as automatic pipettes) can be rich sources of RNases.*
   ** Treat non-disposable glassware and 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, 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 0.1-0.3kb (5S RNA and tRNA), even 4 or 5 bands will appear in some plant tissues. Once the preRNA, hnRNA, small RNA is extracted from the sample, you will see some discontinuous bands of

7kb-15kb. All of them are normal.

5. The most common 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.
6. Prepare chloroform before use.

Ⅴ. Procedure

Note: Add absolute ethanol to Buffer RW and 70% ethanol.

1. Cut sections 5-10μm thick. Immediately place the sections (no less than 50mg) in a 1.5ml microcentrifuge.
2. Add 1ml xylene to the sample, vortex vigorously for 10sec, centrifuge at 12,000rpm for 2min, then discard supernatant.
3. Add 1ml 100% ethanol, centrifuge at 12,000rpm for 2min, discard the supernatant and air dry sample.
4. Add 240μl Buffer PTL, 10μl protease K, and vortex.
5. Incubate at 55℃ for 15min, and then incubate at 80℃ for 15min.
6. Add 750μl Buffer MRL, mix thoroughly, and incubate at RT for 2min.
7. Add 0.2 ml of chloroform. Cap sample tubes securely, Shake tubes vigorously by hand for 15 seconds and incubate them at RT for 3 min.
8. Centrifuge at 12,000rpm for 10 minutes at 4℃;
9. Transfer the aqueous phase (about 600μl) to a new tube. Add 0.6 times volume 70% ethanol. Mix well (precipitate may form). Transfer the mixture and precipitate to a Spin-column RA (placed in collection tube). If the mixture is too much, apply the mixture in successive application to the same Spin-column RA.
10. Centrifuge at 10,000 rpm for 45s at 4℃. Collect the flow-through (including micro RNA)**,** check the volume of flow-through **(please be acute).** Add 100% ethanol (2/3 times volume of flow-through) and mix gently, then put this mixed solution into a Spin-column RB (the volume of RB is about 700μl; one more centrifuge in the same spin-column is needed), centrifuge at 10,000rpm for 30 sec in a microfuge at 4°C and discard flow-through.
11. Add 700μl Buffer RW. Centrifuge at 12,000rpm for 60s. Discard the flow through.
12. Add 500μl Buffer RW. Centrifuge at 12,000rpm for 60s. Discard the flow through.
13. Replace Spin-column RB to the collection tube and spin for 2min to remove the residual fluid.
14. Place Spin-column RB to a 1.5ml RNase-free centrifuge tube. Add 30μl RNase-free H_2O (Pre-heated to 65℃-75℃ is better) to the center of the column RA. Place it at room temperature for 2min. Centrifuge at 12,000rpm for 1min. Keep micro RNA at -20℃ or -80℃.

Ⅵ. Troubleshooting

Problem	Possible Reason	Advices
Low RNA yield	Incomplete lysis and homogenization	Decrease the amount of starting material used, or increase volume of Lysis Buffer. Use the proper homogenization methods according to recommendations in the sample-specific protocols. Cut tissue samples into smaller pieces and ensure the tissue is completely immersed in the Lysis Buffer to achieve optimal lysis.
	Poor quality of starting material	The yield and quality of RNA isolated depends on the type and age of the starting material. Be sure to use fresh sample and process immediately after collection or freeze the sample at –80°C or in liquid nitrogen immediately after harvesting.
	Beyond the binding maximum of silica membrane RNA	Use multiple Spin-columns AC for the same sample.
	Ethanol not added to Wash Buffer RW	Be sure that ethanol was added to Wash Buffer RW
Low A260/280 ratio	Sample was diluted in water. Non-buffered water has variable pH	Use 10 mM Tris-HCl (pH 7.5) to dilute sample for OD measurements.
RNA degraded	RNA contaminated with RNase	Use RNase-free pipette tips with aerosol barriers. Change gloves frequently.
	Improper handling of sample from harvest until lysis	If not processed immediately, quick-freeze tissue immediately after harvesting and store at –80°C or in liquid nitrogen. Frozen samples must remain frozen until Lysis Buffer was added. Perform the lysis quickly after adding Lysis Buffer.

	Tissue very rich in RNases	RNA isolated from tissue rich in RNases may require the addition of RNase inhibitors/inactivators to protect the RNA from degradation, or use a larger volume of Lysis Buffer. Elute samples in 100% formamide. If the RNA is used for mRNA isolation of Northern blots, elute in 0.1% SDS.
Inhibition of downstream enzymatic reactions	Presence of ethanol in purified RNA	Place the Spin column into the Wash Tube and centrifuge the spin column at maximum speed for 2-3 minutes to completely dry the cartridge.