***Note**: for laboratory research use only*

RNA High-purity Total RNA Rapid Extraction Kit

(Spin-column)

Cat. #: **RP1202 (50preps)**

Signalway Biotechnology

I. Kit Content, Storage Condition and Stability

Content	Storage	50 preps (RP1202)
Buffer RL	4°C	55 ml
Buffer RE	RT	30 ml
Buffer RW	RT	15 ml
		Add 60 ml ethanol before use
RNase-free H_2O	RT	10 ml
RNase-free Spin-column AC	RT	50
Collection Tube (2 ml)	RT	50
Buffer RH	RT	9 ml
		Add 21 ml ethanol before use

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

Reminders:

1. ***Please add rational ethanol to Buffer RW and Buffer RH before first use. Mix well and mark the check the box on the label indicting ethanol was added!***
2. All reagents should be clear. Buffers may precipitate due to low temperature, please incubate them at 37°C for a few minutes until it is clear, and then cool down to RT before use.
3. All reagents can be transported under room temperature (15－25℃). Buffer RL can be transported under RT and keep at 4℃ upon arrival.
4. Keep the reagent lids tightly caped when not in use to prevent evaporation, oxidation, and changes in pH.

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 RNA specify 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 RNase-free spin-column, where the total RNA binds to the membrane and contaminants are efficiently washed away. High-quality RNA is then eluted in RNase-free water.

III. Features

- High-purity; specifically membrane absorption and washing for removing protein and other debris.
- Stability, high quality, and high RNA yield. (Up to 100 μg)

IV. Notes

1. **To prevent RNA degradation, all the centrifuge steps should be made under 4**℃ and operated with a traditional centrifuge (with speed up to 13,000 rpm).
2. Buffer RL and Buffer RE contain corrosive compound. Please wear latex gloves to avoid contact with skin, eyes and cloth**. If contact occurs, wash with water or physiological saline.**
3. Due to the prevalence of RNases, wear gloves at all times. 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 plasticware 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. Sometimes there may be the third band about 0.1-0.3 kb (5S RNA and tRNA) and even the fourth or the fifth band (some plant tissue extract). The preRNA, hnRNA, and small RNA appear as bands between 7-15 kb. 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 and RNase-free centrifuge tube before use.

V. Procedure

Note: Add absolute ethanol to Buffer RH

1 Homogenization:

a. Tissues

Please homogenize tissue in an appropriate volume of Buffer RL (50-100 mg/ml) until no visible tissue.

Volume o f sample should not be over 1/10 total volume of Buffer RL.

b. Cells Grown in Monolayer

Append an appropriate volume Buffer RL to the culture plate for dissolve cells and transfer dissolution by pipetting. The volume of Buffer RL is decided by the area of culture plate (about 10 cm^2/1 ml).

If Buffer RL is not enough, genomic DNA will not be completely digested and contaminate the sample.

c. Cells Grown in Suspension

Pellet cells by centrifugation. Lyse cells in Buffer RL by repetitively pipetting. Use 0.75 ml Buffer RL per 5-10 × 10^6 of animal, plant or yeast cells or per 1 × 10^7 bacterial cells.

Washing cells before adding Buffer RL should be avoided. This will increase the possibility of mRNA degradation. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

2 Incubate the homogenized samples for 5 minutes at 15-30°C for a complete dissociation of nucleoprotein complexes.

3 **Optional:** Centrifuge at 12,000 rpm for 10 minutes at 4℃. Pipette the supernatant to an RNase-free centrifuge tube.

An additional isolation step may be required for samples with high content of proteins, fat, polysaccharides or extracellular material such as muscles, fat tissue, and tuberous parts of plants.

4 Add 0.2 ml of chloroform per 1 ml of Buffer RL. Cap sample tubes securely. Shake tubes vigorously by hand for 15 seconds and incubate them at 30°C for 2 to 3 minutes, then centrifuge at 12,000 rpm for 10 minutes at 4℃

5 Transfer the aqueous phase to a new tube. Add 1 volume 70% ethanol. Mix well (precipitate may form). Transfer the mixture and precipitate to an RNase-free Spin-column AC with collection tube (750 μl once).

If the mixture is too much, apply the mixture in successive application to the same Spin-column AC.

6 Centrifuge at 10,000 rpm for 45 seconds at 4℃. Discard the flow through.

7 Add 500 μl Buffer RE to the center of Spin-column AC to remove the protein.

Centrifuge at 12,000 rpm for 45 seconds. Discard the flow through.

8 Add 700 µl Buffer RW. Centrifuge at 12,000 rpm for 45 seconds. Discard the flow through.

9 Add 500 µl Buffer RW. Centrifuge at 12,000 rpm for 45 seconds. Discard the flow through.

10 Replace Spin-column AC to the collection tube and spin for 2 minutes to remove the fluid residual.

11 Place Spin-column AC to a 1.5 ml RNase-free centrifuge tube. Apply 50-80 µl RNase-free H_2O (Pre-heated to 65-75℃) to the center of the column RA. Incubate at room temperature for 2 minutes. Centrifuge at 12,000 rpm for 1 minute.

12 **Optional**: For maximum efficiency, add 30 µl RNase-free water to the center of the column RA for the second elution. Approximately 90% of the RNA is recovered during the first elution step.

13 Store RNA in -20℃ or apply to downstream applications.

VI .Trouble shooting

Problem	Possible Reason	Advices
Low RNA yield	Incomplete lysis and homogenization	Decrease the amount of starting material used or increase volume of Buffer RL. Use the proper homogenization methods. Cut tissue samples into smaller pieces and ensure that the tissue is completely immersed in Buffer RL 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 RA 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 with non-optimal pH	Use 10 mM Tris-HCl (pH 7.5) to dilute sample for OD measurements.
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.
RNA degraded	RNA contaminated with RNase	Use RNase-free pipet tips with aerosol barriers. Change gloves frequently.
	Improperly handling samples	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 Buffer RL is added. Perform the lysis quickly after adding Buffer RL.

	Tissue is rich of RNases	RNA isolated from RNase-rich tissues may require the addition of RNase inhibitors/inactivators to protect the RNA from degradation. Use a larger volume of Buffer RL. If the RNA is used for Northern blots, elute in 0.1% SDS.
	RNA contaminated with RNase	Use RNase-free pipet tips with aerosol barriers. Change gloves frequently.