*Note: for laboratory research use only*

# RNApure High-purity Total RNA Rapid Extraction Kit

## (Spin-column）

**Cat.#:** **RP 1201(20preps)**
**RP1202 (50preps)**

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## Ⅰ. Kit Content, Storage Condition and Stability

| Content | Storage | 20preps （RP1201） | 50preps （RP1202） |
|---|---|---|---|
| Buffer RL | 4℃ | 25 ml | 55ml |
| Buffer RE | RT | 15ml | 30 ml |
| Buffer RW | 4 (half 1 year)<br>-20 (long term) | 6 ml<br>***Add ethanol before use*** | 15ml |
| RNase-free $H_2O$ | RT 5ml | | 10ml |
| RNase-free Spin-column AC | RT 20 | | 50 |
| Collection Tube（2ml） | RT | 20 | 50 |
| 70% ethanol | RT | 4ml RNase-free $H_2O$<br>***Add ethanol before use*** | 9ml RNase-free $H_2O$ |

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

## Notes:

1. ***Please add ration ethanol to Buffer RW and 70% ethanol before first use. 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. In case, some may precipitate due to low temperature, please incubate them at 37 for a moment **until clear**, and then cool down to RT before use.
3. Some reagents will precipitate because of been stored in 4℃ or - 20℃, which will affect the using effect, so incubate till no pricipitation before use. All reagents can be transported under room temperature (15℃ — 25℃). Buffer RL can be transported under RT and keep at **4℃ upon arrival.**
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 Mini spin-column, where the total RNA binds to the membrane and contaminants are efficiently washed away. High-quality RNA is then eluted in 30–100 µl water.

## Ⅲ.Features

- Stability, comparable RNA yield with high quality adsorbing membrane.
- High-purity, specifically 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℃, except having special notes**, suggest using up to 13,000 rpm traditional centrifuge, for example Eppendorf 5415C.
2. Buffer RL and Buffer RE contain stimulating compound, please wear latex gloves, avoiding skin, eyes and cloth to be contaminated. **If that, please use water or physiological saline to wash the exposed body parts.**
3. Due to the prevalence of RNases, wear gloves at all times and change them whenever may have been contacted by reagents, 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 it is 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 0.1-0.3kb (5S RNA and tRNA), even 4 or 5 bands will appear in some plant tissues. Once the preRNA, hnRNA, small RNA are extracted from the sample, you will see some discontiguous 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 WB and 70% ethanol.***

1 Homogenization:

*a. Tissues*

*Please homogenize tissue in an appropriate volume of Buffer RL (50-100mg/mL) until no visible tissue. Pay attention to the volume of sample should not beyond 1/10 total volume of Buffer RL.*

*b. Cells Grown in Monolayer*

*You can directly append an appropriate volume Buffer RL to the culture plate for dissolve cell, and transfer dissolution by pipetting. The volume of Buffer RL is decided by the area of culture plate, about 10cm$^2$ per 1ml. Once appending not enough Buffer RL, it's possible to contaminated genomic DNA.*

*c. Cells Grown in Suspension*

*Pellet cells by centrifugation. Lyse cells in RL by repetitive pipetting. Use 0.75 ml of the reagent per 5-10 × 10$^6$ of animal, plant or yeast cells, or per 1 × 10$^7$ bacterial cells. Washing cells before addition of RL should be avoided as this increases 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 to permit the complete dissociation of nucleoprotein complexes.

3 **Optional:** Centrifuge at 12,000 rpm for 10 min at 4℃. Pipette the supernatant to a 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.

5 Centrifuge at 12,000rpm for 10 minutes at 4℃;
(Centrifuge the samples at no more than 12,000 rpm for 15 minutes at 2~8℃).

6 Transfer the aqueous phase to a fresh tube. Add 1 volume 70% ethanol. Mix well (precipitate may form). Transfer the mixture and precipitate to a Spin-column AC (placed in collection tube). If the mixture is too much, apply the mixture in successive application to the same Spin-column AC.

7 Centrifuge at 10,000 rpm for 45s at 4℃. Discard the flow through. Reuse the Spin-column and the collection tube.

8 Add 500μl Buffer RE to the center of Spin-column AC to remove the protein. Centrifuge at 12,000 rpm for 45s. Discard the flow through.

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

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

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

12 Place Spin-column AC to a 1.5ml RNase-free centrifuge tube. Apply 50-80μl 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. If desired, wash the Spin-column AC with 30μl RNase-free water, combining the

second eluate with the first in the same Collection Tube; approximately 90% of the RNA is recovered during the first elution step.

## 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 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 maxium 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. 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 pipet 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. |