

Blood/Liquid sample Total RNA Rapid Extraction Kit (Spin-Column)

RP 4001 (50 preps)

Kit Content, Storage Condition and Stability

Content	Storage	50 preps
Buffer RLS	4°C	55 ml
Buffer RE	RT	30 ml
Buffer RW	RT	15 ml
		Add the ration ethanol before use
RNase-free H ₂ O	RT	10 ml
RNase-free Spin Column AC	RT	50
Collection Tubes 2 ml	RT	50
70% ethanol	RT	9 ml RNase-free H ₂ O
		Add the ration ethanol before use

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

Note:

Please add ration ethanol to Buffer RW and 70% ethanol before use. Mix well and mark the check box labeled on the bottles to indicate that the ethanol has been added.

All reagents should be clear. In case, some may precipitate due to low temperature, please incubate them at 37°C for a moment until clear, and then cool down to RT before use.

Some reagents will precipitate because of been stored in 4°C or -20°C, which will affect the using effect, so incubate till no precipitation before use. Buffer RLS can be transported under room temperature (15°C-25°C) and kept at 4°C upon arrival.

Please ensure the bottles tightly capped when not in use, prevent reagents from evaporating, oxidation and pH change.

Principle

The RNzol LS procedure represents a well-established technology for RNA purification. This technology combines the selective binding properties of a silica-based membrane with the speed of microspin technology. A specialized high-salt buffer system allows up to 100 µg of RNA longer than 200 bp binding to the RNzol LS 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 purification of intact RNA. Ethanol is added to provide appropriate binding conditions and the lysate is transferred to the 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.

This kit can be used to isolate RNA from whole blood, biological fluids and other liquid samples.

In addition, Lysis Buffer RLS is a great storage solution for total RNA. Lysate (add Lysis Buffer RLS to sample based on the volume ratio 3:1 and vortex rigorously) can be stored up to 2 months at -20°C and half year at -80°C; also the lysate can be stable up for 1 day at 4°C and 1 week at -20°C for transportation.

Features

- Convenience, no need to lyse erythrocytes and separate leukocytes for isolation total RNA from whole blood.
- Stability, comparable RNA yield with high quality absorbing membrane.
- High-purity, specific membrane absorption and wash-elution system ensure to remove protein and other debris.

Notes

- 1. To prevent RNA degradation, all the centrifugation steps should be performed under 4°C, except special notes;** suggest using up to 13,000 rpm traditional centrifuge, for example Eppendorf 5415C and the similar.
- Buffer PL 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.**
- 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 Cloning" rules.
 - * Wear gloves in entire process. Skin often contains bacteria and molds that can contaminate RNA preparation and are 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.*
- 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), 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 7 kb-15 kb. All of them are normal.
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- The most common method to determine the yield and purity of RNA is spectrophotometry (OD260/OD280). Please dissolve RNA by TE, water will make OD280 higher because of lower ion intensity and pH.

Procedure

Note: Add absolute (100%) ethanol to the bottles Wash Buffer WB and 70% ethanol, based on the instructions on the bottle labels before starting.

- 1. Pipet 750 µL Lysis Buffer RLS to a 1.5 mL microcentrifuge tube. Add 250 µL whole blood, biological fluids or other liquid samples to the Lysis Buffer RLS in the microcentrifuge tube. Vortex for 2 min to mix thoroughly.**
 - If the sample volume is larger than 250 µL, increase the amount of Lysis Buffer RLS proportionally (e.g., a 500 µL sample will require 1500 µL Lysis Buffer RLS).
 - Generally, plasma, serum and other body fluids often have low RNA. Hence, to concentrate these samples is recommended. Please follow the manufacturer's instructions to concentrate samples to the final volume 250 µL.
2. Incubate for 10 minutes at RT.
3. Add 150 µL chloroform and shake tubes vigorously for 15 sec and incubate for 3 min at RT.
4. Centrifuge the samples at 12,000 rpm for 10 min at 4°C. The mixture separates into 3 phases: an upper aqueous phase, interphase and a lower phenol-chloroform phase. RNA remains in the upper aqueous phase. The volume of aqueous phase is around 60% of Lysis Buffer RLS for homogenization.
5. Transfer the aqueous phase to a fresh tube, add 500 µL 70% ethanol.
6. Place the Spin-column AC to the Collection Tube, transfer the alcohol-aqueous mixture to the Spin-column AC, centrifuge at 10,000 rpm for 1 min and discard the filtrate.

7. Place the Spin-column AC back to Collection Tube; add 500 µl Protein Precipitation Buffer RE to Spin-column AC, centrifuge at 10,000 rpm for 1 min, and discard the filtrate.
8. Place the Spin-column AC back on the Collection Tube, add 700 µl Washing Buffer RW, centrifuge at 12,000 rpm for 1 min and discard the filtrate.
9. Add 500 µl Washing Buffer RW, centrifuge at 12,000 rpm for 1 min and discard the filtrate.
10. Place the Spin-column AC to the Collection Tube and spin for 2min to remove the residual fluid.
11. Place the Spin-column AC to a 1.5ml RNase-free centrifuge tube. Add 50-80 µl RNase-free H₂O (better if pre-heated to 65°C -75°C) to the center of the Spin-column. Place at room temperature for 2 min. Centrifuge at 12,000 rpm for 1 min. If desired, wash the Spin-column with 30 µl RNase-free water, combining the second eluate with the first in the same tube; approximately 90% of total RNA is recovered during the first elution step.
13. Store total RNA at -20°C and -80°C for long-term storage.

Troubleshooting

Problem	Possible Reason	Advices
Low DNA/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 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.