

Note: *for laboratory research use only*



Liquid Blood Total RNA Rapid Extraction Kit
(Spin-column)

Cat.# : RP 4001(50 preps)

RP4002 (100 preps)


ABigen Corporation

I. Kit Content, Storage Condition and Stability

Content	Storage	50 preps (RP4001)	100 preps (RP4002)
Buffer RLS	4°C	55 ml	110 ml
Buffer RE	RT	30ml	60ml
Buffer RW	RT	15 ml	25 ml
		<i>Add ethanol before use.</i>	
RNase-free H ₂ O RT		9ml	9ml×2
RNase-free Spin-column AC	RT 50		100
Collection Tube (2ml)	RT 50		100
70% ethanol	RT	RNase-free H ₂ O 9ml	RNase-free H ₂ O 9ml ×2
		<i>Add ethanol before use.</i>	

All reagents are stable up to 12 months when stored properly.

Notes:

1. Please add proper volume ethanol to the bottles labeled Buffer RW and 70% ethanol before starting. Mix well and check bottles to indicate that the ethanol has been added.
2. All reagents should be clear. In case, some may form precipitate due to low temperature, please incubate them at 37 for a moment **until clear**, and then cool to RT before use.
3. Some reagents will form precipitation, which affects RNA yields, under improper low temperature (4°C or -20°C). Please transport and keep reagents all at room temperature (15°C – 25°C). Buffer RLS can be transported under RT and kept at 4°C **upon arrival**.
4. Please ensure the bottles of buffer tightly capped when not in use, preventing reagents evaporating, oxidation and pH change.

II. Principle

This 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 200bp to bind to the 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 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 in transportation.

III. 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.

IV. 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.
2. 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.**
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 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 dissolve RNA by TE, water will make OD_{280} higher because of lower ion intensity and pH.

V. Procedure

Note: Add the proportion volume of 100% ethanol to the bottles labeled Wash Buffer WB and 70% ethanol based on the instruction in the bottles before starting.

1. **Pipet 750μL Lysis Buffer RLS to a 1.5mL microcentrifuge tube. Add 250μL whole blood, biological fluids, or other liquid samples to the Lysis Buffer RLS in the microcentrifuge tube. Vortex for 2min to mix thoroughly.**
If the sample volume is larger than 250μL, increase the amount of Lysis Buffer RLS proportionally (e.g., a 500μL sample will require 1500μL 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 250μL.
2. **Incubate for 10min at RT.**
3. **Add 150μL chloroform and shake tubes vigorously for 15sec, and incubate for 3min at RT.**
4. **Centrifuge the samples at 12,000 rpm for 10min 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 500μL 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 1min, and discard the filtrate.**
7. **Place the Spin-column AC back to Collection Tube; add 500μl Protein Precipitation Buffer RE to Spin-column AC, centrifuge at 10,000 rpm for 1min, and discard the filtrate.**
8. **Place the Spin-column AC back on the Collection Tube, add 700μl Washing Buffer RW, centrifuge at 12,000 rpm for 1min, and discard the filtrate.**
9. **Add 500μl Washing Buffer RW, centrifuge at 12,000 rpm for 1min, 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 50-80µl RNase-free water (pre-heated to 65°C-75°C is better) to the center of the Spin-column AC. Leave 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 tube; approximately 90% of the RNA is recovered during the first elution step.

VI. Troubleshooting

Problem	Possible Reason	Solutions
Low RNA yield	Incomplete lysis and homogenization	Decrease the amount of starting material used, or increase volume of Lysis Buffer. 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 silicified membrane RNA	Using multiple spin-columns for the same sample.
	Ethanol not added to Wash Buffer RW	Be sure that ethanol was added to Wash Buffer RW
Low A _{260/280} ratio	Sample was diluted in water nonbuffered water has variable pH	Use 10 mM Tris-HCl (pH 7.5) to dilute sample for OD measurements.

RNA degraded	RNA contaminated with RNases	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.
	RNase- rich tissues	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 enzyme digestion	Presence of ethanol in purified RNA	Place the spin-column into the collection tube and centrifuge the spin-column at maximum speed for 2-3 minutes to completely dry the cartridge.