Note: for laboratory research use only.

Universal Plant Total RNA Extraction Kit (Spin-column)

Cat.# RP 3301(50 preps)

RP3302 (100 preps)

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Ⅰ. Kit Component, Storage Condition and Stability

Content	Storage	50 preps （RP3301）	100 preps （RP3302）
Buffer PL	RT	55ml	110 ml
Buffer RE	RT	30 ml	60 ml
Buffer RW	4 one month /-20 up to one year	15ml 25 ml	
		Add the ration ethanol before use.	
RNase-free H_2O	RT 10ml		20 ml
RNase-free Filtration-column	RT 50		100
RNase-free Spin-column AC	RT	50	100
Collection Tube （2ml）	RT	100	200
70% ethanol	RT	22.5 ml RNase-free H_2O	45 ml RNase-free H_2O
		Add the ration ethanol before use.	

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 use. Mix well and make a check mark in the empty 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 to RT before use.
3. Some reagents will form precipitation, which affects RNA quality and yield, under improper low temperature （4℃ or - 20℃）. Please transport and keep all reagents at room temperature (15℃-25℃). Buffer RL can be transported under RT and kept at **4**℃ **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 unique product, different with phenol /guanidine isothiocyanate method, is explored by BioTeke, applied to extract RNA from plant sample that TRIZOL and RNeasy Mini Kit do not work. It can effectively separate RNA with polysaccharides and easily remove polyphone. There are about 100 species of plant samples having been confirmed with improvement in RNA recovery ratio by using this product.

Ⅲ. Features

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

Ⅳ.Notes

1. **To prevent RNA degradation, all the centrifugation steps should be made under 4℃, except special notes**, suggest use up to 13,000 rpm traditional centrifuge, for example Eppendorf 5415C and the similar.
2. Buffer PL and Buffer RE containing stimulating compound, please wear latex gloves, avoiding skin, eyes and cloth to be contaminated. **If that, please wash with water or physiological saline.**
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 dissolute RNA by TE, water will make OD_{280} higher because of lower ion intensity and pH.

Ⅴ.Procedure

Note: Add 100% ethanol to the bottles labeled Wash Buffer WB and 70% ethanol.

Pre-heat Lysis Buffer PL to 65℃ before use.

1. Homogenize samples in 10–20 volumes Lysis buffer PL (e.g., 1 mL Lysis buffer PL per 50-100 mg tissue) using standard homogenization procedures. For most tissues, rotor-stator homogenizers are very effective.
2. Transfer the sample to a RNase-free 1.5ml centrifuge tube and add 1ml Lysis Buffer PL. Mix well and incubate at 65℃ for 5min.
3. Centrifuge at 12,000rpm for 10min at 4℃. Pipette the supernatant to an RNase-free Filtration-column **(to remove genomic DNA)**.
4. Centrifuge at 12,000rpm for 10min at 4℃ Collect the flow through to a 1.5ml RNase-free centrifuge tube.
5. Add 1 volume 70% ethanol .Mix well (precipitate may form). Transfer the mixture and precipitate to a Spin-column AC (place in collection tube, to **absorb total RNA**). If the mixture is too much, apply the mixture in successive application to the same Spin-column AC.
6. Centrifuge at 10,000rpm for 45s at 4℃. Discard the flow through. Reuse the Spin-column AC and the collection tube
7. Add 500μl Buffer RE to the center of the Spin-column AC to remove the protein. Centrifuge at 12,000rpm for 45s. Discard the flow through.
8. Add 700μl Buffer RW. Centrifuge at 12,000rpm for 45s. Discard the flow through.
9. Add 500μl Buffer RW. Centrifuge at 12,000rpm for 45s. Discard the flow through.
10. Replace 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. Apply 50-80μl RNase-free water (Pre-heated to 65℃-75℃ 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 Collection Tube; approximately 90% of the RNA is recovered during the first elution step.

Ⅵ.Troubleshooting

Problem	Possible Reason	Advices
Low RNA yield	Incomplete lysis and homogenization	Decrease the amount of starting material, 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 or in liquid nitrogen immediately after harvesting.
	Beyond the binding maximum of silicified membrane RNA	Using multiple Spin-columns AC for the same sample.
	Ethanol not added to Buffer RW	Be sure that ethanol was added to Buffer RW.
Low A260/280 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 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 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 Collection Tube and centrifuge the spin-column at maximum speed for 2-3 minutes to completely dry the cartridge.

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