

Universal Plant Total RNA Rapid Extraction Kit

(Spin-column) – type 2

A fast kit for the isolation of total RNA from plants

For laboratory research use only

RP3311 50 preps

Kit Content, Storage, and Stability

Content	Storage	50 preps
Buffer PL	RT	55 ml
Buffer RE	RT	30 ml
Buffer RW	4°C one month/-20°C one year	15 ml
	Add ration ethanol before first using	
RNase-free water	RT	10 ml
RNase free Spin Column AC	RT	50
Collection Tube (2 ml)	RT	50
70% ethanol	RT	22,5 ml RNase-free H ₂ O
	Add ration ethanol before first using	

All reagents are stable for 12 months when stored properly.

Please add proper volume ethanol to the bottles labeled Buffer RW and 70% ethanol before starting. Mix well and make a check mark in the empty box labeled on the bottles to indicate that the ethanol has been added.

Please prepare β -mercaptoethanol and phenol:chloroform:isoamylalcohol (25:24:1) before starting. Please make this mixture with water saturated phenol.

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

Some reagents will form precipitation, which affects RNA quality and yield, under improper low temperature (4°C or -20°C). Please transport and keep all reagents at room temperature (15-25°C). Buffer RL can be transported under RT and kept at 4°C upon arrival.

Please ensure the bottles of buffer tightly capped when not in use, preventing reagents evaporating, oxidation and pH change.

Principle

This unique product, different with phenol /guanidine isothiocyanate method,, applies to extract RNA from plant samples that TRIZOL and RNeasy Mini Kit do not work well. It can effectively separate RNA with polysaccharides and easily remove polyphene. There are about 100 species of plant samples having been confirmed with improvement in RNA recovery ratio by using this product.

Features

Stability, comparable RNA yield with high quality absorbing membrane.

High-purity, specifically membrane absorption and washing for removing protein and other debris

β -mercaptoethanol and phenol:chloroform:isoamylalcohol (25:24:1) has been used to remove genomic DNA and proteins.

Notes

To prevent RNA degradation, all the centrifugation steps should be made under 4°C, except special notes, suggest use up to 13,000 rpm traditional centrifuge.

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.**

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.*

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.

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.

Procedure

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

Pre-heat Lysis Buffer PL to 65°C before use.

Add β -mercaptoethanol to Lysis Buffer PL to final concentration 1% that is 10 μ l β -mercaptoethanol per 1 ml of Lysis Buffer PL

1. Take around 100mg tissues and grind with liquid nitrogen.

Optional: Homogenize sample in 10 volumes Lysis buffer PL (e.g., 1 mL Lysis buffer PL per 50-100 mg tissues) if there is no liquid nitrogen.

2. Transfer the sample to an RNase-free 1.5ml centrifuge tube and add 1 ml Lysis Buffer PL (**Check if β -mercaptoethanol added!**), mix well and incubate at **65 °C for 5 min**.

3. Centrifuge at 12,000 rpm for 10 min at 4°C. Pipette the supernatant to an RNase-free 1.5 ml centrifuge tube.

4. Add equal volume of phenol:chloroform:isoamylalcohol (25:24:1), vortex for 15 sec, incubate at RT for 3 min.

5. Centrifuge at 12,000 rpm for 5 min at 4°C. The solution separates into 3 phases: an upper aqueous phase, interphase and a lower phenol-chloroform phase. RNA remains in the upper aqueous phase.

6. Transfer the upper aqueous phase to an RNA-free centrifuge tube, and add equal volume 70% ethanol (**check if 100% ethanol added!**). Mix well (precipitate may form). Transfer the mixture and precipitate to a Spin-column AC (placed in collection tube, **to absorb total RNA**). If the volume of mixture is more than 700 μ l, please elute twice through the same Spin-column AC.

7. Centrifuge at 10,000 rpm for 45 sec at 4°C. Discard the flow through. Reuse the Spin-column AC and the collection tube.

8. Add 500 μ l Buffer RE to the center of the Spin-column AC to remove the proteins. Centrifuge at 12,000 rpm for 45 sec. Discard the flow through.

9. Add 700 μ l Buffer RW (check if 100% ethanol added!). Centrifuge at 12,000 rpm for 45 sec. Discard the flow through.

10. Add 500 μ l Buffer RW. Centrifuge at 12,000 rpm for 45 sec. Discard the flow through.

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

12. Place the Spin-column AC to a 1.5ml RNase-free centrifuge tube. Apply 50-80 μ l 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 2 min. Centrifuge at 12,000 rpm for 1 min. If desired, wash the Spin-column AC with additional 30 μ l 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

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°C 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°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 Collection Tube and centrifuge the spin-column at maximum speed for 2-3 minutes to completely dry the cartridge.