***Note**: for laboratory research use only*

Universal Plant Total RNA Extraction Kit (Spin-column) - I

Cat. #: DP3301 (50 preps)

Signalway Biotechnology

Ⅰ.Kit Content, Storage and Stability

Content	Storage	50 preps (DP3301)
Buffer PL	RT	55 ml
Buffer RE	RT	30 ml
Buffer RW	4℃ for 6 months －20℃ for 1 year	15 ml
		Add 60 ml ethanol before first use
70% ethanol	RT	22.5 ml
RNase-free H_2O	RT	10 ml
RNase-free Spin-column AC	RT	50
Collection Tube (2ml)	RT	100
RNase-free Filtration-column	RT	50

All reagents are stable for 12 months when stored properly.

Reminder:

1. ***Add ethanol to Buffer RW before use, mix adequately, and then check the box on the label showing ethanol was added!***
2. Some reagents may precipitate under low temperature. Incubate at 37°C for a few minutes **until buffer is clear**, and then let the buffer cool to RT before use. Buffer PL can be transported under RT and kept at **4°C upon arrival**.
3. Keep the reagent lids tightly caped when not in use to prevent evaporation, oxidation, and changes in pH.

Ⅱ. Principle:

This kit can effectively separate RNA with polysaccharides and easily remove polyphone, which applied to extract RNA from plant sample that TRIZOL and RNeasy Mini Kit do not work. There are about 100 species of plant samples having been confirmed with improvement in RNA recovery ratio by using this product. After a serial of elution-centrifugation steps to remove cellular metabolite and proteins,

RNA selectively binds to silica membrane. Total RNA is eluted from silica membrane in low salt RNase-free water.

Ⅲ. Features:

1. Rapid and convenient. Do not contain poisonous phenol and do no require ethanol precipitation.
2. The purified RNA without any protein contamination can be applied for Northern Blot, Dot Blot, mRNA Isolation, cDNA Synthesis, Primer Extension, mRNA differential Display etc.

Ⅳ. Notes

Please read this section before your experiment.

1. **To prevent RNA degradation, all the centrifugation steps should be performed under 4°C unless being specially noted**. Recommend using traditional centrifuge (with up to 13,000 rpm), for example Eppendorf 5415C or the similar machine.
2. Buffer PL and Buffer RE contains a corrosive compound; wear latex gloves to avoid direct contact with skin, eyes, and clothes. If contact occurs, wash with water or physiological saline.
3. Due to the prevalence of RNases, wear gloves during the procedure and change them whenever contact by reagents occurs; please follow standard laboratory procedures of "Molecular Clone" rules.

   * *Wear gloves during entire process. Skin often contains bacteria and molds that can contaminate an RNA preparation and be a source of RNases.*
   * *Use sterile, disposable plastic-ware, 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*

non-disposable items (such as automatic pipettes) can be rich sources of RNases.

* *Treat non-disposable glassware and non-disposable plastic-ware before use to ensure that they are RNase-free. Bake glassware at 200°C overnight, and thoroughly rinse plastic-ware with 0.1N NaOH with 1 mM 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. There may be the third band about 0.1-0.3kb (5S RNA and tRNA) and even 4 or 5 bands; the preRNA, hnRNA, and small RNA appear as bands between 7-15 kb. All of them are normal.

1. The most common method to determine the yield and purity of RNA is using spectrophotometer (OD_{260}/OD_{280}). Dissolute RNA by TE (water will make OD_{280} higher because of lower ion intensity and pH).

Ⅴ. Procedure

Before Starting

- ***Dilute Buffer RW and 70% ethanol with absolute ethanol, vortex adequately, then mark the check box, avoid multi-adding!***
- ***Pre-heat Buffer PL to 65°C before use***

1. Homogenize samples in 10–20 volumes buffer PL (1 mL 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 RNase-free 1.5 ml centrifuge tube and add 1 ml Buffer PL. Mix thoroughly and incubate at 65℃ for 5 min.
3. Centrifuge at 12,000 rpm for 10 min at 4℃. Pipette the supernatant to an RNase-free Filtration-column **(to remove genomic DNA)**.
4. Centrifuge at 12,000 rpm for 10 min at 4℃ and transfer the flow-through to a 1.5 ml RNase-free centrifuge tube.
5. Add 1 volume 70% ethanol and 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, repeat loading mixture in successive application to the same Spin-column AC.

6. Centrifuge at 10,000 rpm for 45 seconds at 4℃. Discard the flow-through. Reuse the Spin-column AC and the collection tube
7. Add 500 µl Buffer RE to the center of the Spin-column AC to remove the protein. Centrifuge at 12,000 rpm for 45 seconds. Discard the flow-through.
8. Add 700 µl Buffer RW. Centrifuge at 12,000 rpm for 45 seconds. Discard the flow-through.
9. Add 500 µl Buffer RW. Centrifuge at 12,000 rpm for 45 seconds. Discard the flow-through.
10. Replace the Spin-column AC to the collection tube and spin for 2 min to remove the residual fluid.
11. Place the Spin-column AC to a 1.5 ml RNase free centrifuge tube. Apply 50-80 µl RNase-free water (Pre-heated to 65-75℃) to the center of the Spin-column AC. Cool down to room temperature for 2 min. Centrifuge at 12,000 rpm for 1 min.
12. (Optional) add additional 30 µl RNase-free water in the spin-column, and cool down to room temperature for 2 min. Centrifuge at 12,000 rpm for 1 min.

    The volume of elution buffer could be adjusted according to needs. Appropriately reduce elution volume can increase concentration. The minimum volume is 30 µl (Less than 30 µl will decrease the elution efficiency and the RNA yield).

13. Keep DNA at -20℃ or apply to down-stream reactions.

Ⅶ. Troubleshooting

Problem	Possible Reason	Advices
Low RNA yield	Incomplete lysis and homogenization	Decrease the amount of starting material, or increase volume of Buffer PL. Cut tissue samples into smaller pieces and ensure the tissue is completely immersed in the Buffer PL 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 or 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 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 Buffer PL was added. Perform the lysis quickly after adding Buffer PL.
	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 Buffer PL. 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.