Gene-Spin Plant Total RNA Purification Kit

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For research use only

Cat#：PT-RNA-PL-50

Size：50 rxns

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Product Introduction:

The Plant Total RNA Purification Kit represents a simple and convenient approach for preparation of high-purity total RNA from a wide variety of plant samples. By utilizing silica membrane column, this kit offers a variety advantages, including eliminates the requirement of ethanol precipitation and the usage of organic solvent such as phenol and chloroform. In addition, the kit also contains two kind of different lysis buffers, guanidine thiocyanate and SDS with anti-oxidants, which could process most plant and fungal samples. Plant tissues are first homogenized and lysed in the presence of RNA lysis solution A or B by different protocols and disruption conditions. After homogenization, the sample is then processed through a miniprep column and the RNA is bound to the silica membrane for further washing and desalting. Highly purified and full-length total cellular RNA is then eluted in a small volume of nuclease-free water and is ready for use in any downstream application. This kit could not efficiently purify the RNA which is shorter than 200 nucleotides including tRNA, 5S RNA, 5.8S RNA and microRNA.

Suggestions for Plant RNA Lysis Solution:

Because cellular structure and metabolic activity of plants can vary significantly between species, tissue type and developmental stages, there is no universal lysis solution or unique protocol that can be applied equally well to all plant samples.

This kit includes two RNA Lysis buffers, RNA Lysis Solution A and B. The Plant RNA Lysis Solution A is based on guanidinium thiocyanate, which works with many plant tissues, including pineapple leaves and orchid leaves, which Solution B does not work well with. The protocol for Solution A is easier and faster than the one for Solution B, but yield is generally lower than when using Solution B. Solution A does not work with some plant tissues containing high amounts of starch, polyphenols and secondary metabolites (such as some woody trees, milky endosperm, tubers or mycelia of filament fungi). Therefore, these plant tissues cannot be used with this buffer because Solution A will become solidified or very viscous after being

added to the sample.

The Plant RNA Lysis Solution B is based on the SDS/anti-oxidant method, which works best with the broadest range of plant tissues, including herbaceous and woody plant leaves, very viscous samples rich in polyphenols, polysaccharides and seed endosperm. The yield for Solution B is generally higher (up to 40%) than for Solution A, but Solution B works less efficiently with pineapple and orchids than Solution A.

Nevertheless, both solutions can work with most samples. However, when higher yield or removal of polysaccharides and polyphenols is required, RNA Lysis Solution B should be the buffer of choice.

Summary of RNA Lysis Solution:

	RNA Lysis Solution A	RNA Lysis Solution B
Major Ingredient	guanidinium thiocyanate	SDS/anti-oxidant
Application	Most plant tissues, **BUT NOT** for high starch, polyphenols and polysaccharide samples.	Broadest range of plant tissues including high starch, polyphenols and polysaccharide samples, **NOT** for orchid leaves and pineapple leaves.
Ease of Use	Easier than Lysis Solution B	Needs extra steps to remove polyphenols and polysaccharides
Yield and Quality	Equivalent to most other suppliers	Generally higher than of Solution A and other suppliers

Kit Contents:

Reagents/Tubes provided	
Plant RNA Lysis Solution A	30 mL
Plant RNA Lysis Solution B *	30 mL
DNase I Solution 2 U/μl	120 μL
DNase I Incubation Buffer *	4.5 mL
Protein Precipitation Solution	10 mL
RNA Binding Solution	15 mL
RNA Wash Solution I	55 mL
RNA Wash Solution II	16 mL
Nuclease-free Water	10 mL
RNA Spin Filter with Collection Tube	50 preps
RNA Spin Column with Collection Tube	50 preps

Storage & Stability:

* Please store the Plant RNA Lysis Solution B and DNase I Solution at -20°C; store all other kit components at room temperature (25~28°C). All buffers are stable for a period of at least 12 months from the date of receipt when stored under ambient conditions. Please avoid exposure to direct sunlight or extremes in temperature.

Preparation before experiment:

1. If working with RNA Lysis Solution A, add 300 μl of 2-Mercaptoethanol (2-ME) to the RNA Lysis Solution A, and store RNA Lysis A/2-ME Solution at 4°C.

2. If working with RNA Lysis Solution B, thaw the Plant RNA Lysis

Solution B in a water bath at 50°C to completely dissolve it. Then add 450 µl of 2-Mercaptoethanol (2-ME) to the RNA Lysis Solution B, and store RNA Lysis B/2-ME Solution at 4°C.

3. If working with RNA Lysis Solution B, add 30 ml 100% Ethanol into the RNA Binding Solution and mix well.
4. Add 64 ml of 100% Ethanol to the RNA Wash Solution II.

Note: For the best quality and yield of RNA, harvest plant tissues immediately before you start the procedure. Alternately, tissues may be immediately snap-frozen in liquid nitrogen and stored at -70°C, or tissues can be stabilized in RNA Keeper™ reagent (Cat. No. PT-R485). The plant tissue sample can be stored in RNA Keeper™ reagent for a day at 37°C, 1 week at room temperature, one month at 4°C and indefinitely at -20°C.

Note: For some homogenates from plant tissues which are very viscous and glue-like, such as those of the *Lauraceae* family, please follow the modified protocol in **Appendix I** (page 14).

Equipment / Reagents required but not provided:

- Mortar and pestle
- 100% Ethanol: For preparing the RNA Binding Solution and RNA Wash Solution II
- 2-Mercaptoethanol (2-ME) : For preparing the RNA Lysis Solution
- Water bath or heating block at 60°C

Protocol with RNA Lysis Solution A

1. Quickly weigh ≤ 100 mg of fresh tissue, either frozen or RNA Keeper^{TM} reagent stabilized, and homogenize it completely under liquid nitrogen.
   ** Do not allow the frozen sample to thaw during weighing before adding the Plant RNA Lysis A/2-ME Solution.*

2. Immediately transfer the homogenate (<100 mg) into a microcentrifuge tube and add 450 µl of Plant RNA Lysis A/2-ME Solution, vortex vigorously and then incubate at 60°C for 3 min. Centrifuge the tube at top speed (12,000~14,000 x*g*) for 5 min.

3. Transfer the supernatant into a RNA Spin Filter (with yellow ring) inserted in a clean 2 ml Collection Tube. Spin at top speed for 2 min.
   ** Most tissue clumps and cell debris can be removed by Spin Filter, but a small amount will pass through the filter and form a pellet in the collection tube. Do not disturb the pellet.*

4. Transfer the flow-through from the collection tube into a new 1.5 ml microcentrifuge tube, avoiding disrupting and pipetting the pellets.

5. Add 0.5 volumes (about 225 µl) of 100% Ethanol to the lysate, and mix well by pipetting.
   ** Do not centrifuge after Ethanol is added.*

6. Load the lysate/ethanol mixture (<700 µl) into the RNA Spin Column inserted in a 2 ml collection tube, spin at top speed for 1 min, and discard the flow-through.
   ** If the volume of lysate/ethanol mixture is greater than 700 µl, apply any remaining lysate/ethanol mixture into the RNA spin column and repeat centrifugation step as above.*

7. Place the RNA spin column to the original collection tube and add 500 µl of RNA Wash Solution I, spin at top speed for 1 min and discard the flow-through.

8. **DNase I digestion.**
   For each isolation, premix 80 µl DNase I Incubation Buffer with 2 µl DNase I in a microcentrifuge tube (mix by flicking or inverting the tube, do not vortex!). Add 82 µl of the solution to the center of RNA spin column membrane and incubate it at room temperature for 15 min.
   ** If processing multiple samples at a time, prepare a fresh master mix of DNase I solution before use; do not store a premix of DNase I solution.*

9. Add 500 µl of RNA Wash Solution I to the RNA Spin Column, spin at top speed for 1 min and then discard the flow-through.

10. Place the RNA Spin Column into the original collection tube and add 600 µl of RNA Wash Solution II, spin at top speed for 1 min and then discard the flow-through. Repeat this step once more.

11. Place the RNA Spin Column onto the original collection tube and spin at top speed for 3 min to remove any residual ethanol from RNA Wash Solution II used in the previous step. Transfer the RNA spin column in a clean 1.5 ml microcentrifuge tube.
   ** If centrifugation speed is lower than 12,000 xg or residual ethanol from RNA Wash Solution II must be removed completely, incubate the RNA spin column at 60~65°C in a drying oven for 5 min to evaporate all of the remaining ethanol.*

12. Add 30~50 µl **Nuclease-free water** into the center of the RNA Spin Column membrane and let stand for 1 min. Centrifuge at top speed for 1 min and store the RNA sample at -70°C.

<u>**Protocol with RNA Lysis Solution B**</u>

1. Quickly weigh no more than 100 mg of fresh tissue, or frozen or RNA KeeperTM stabilized tissue, and homogenize it under liquid nitrogen.

   ** Do not allow the frozen sample to thaw during weighing before adding the Plant RNA Lysis B Solution.*

2. Immediately transfer the homogenate (<100 mg) to a microcentrifuge tube and add 450 μl of Plant RNA Lysis Solution B/2-ME, vortex vigorously and incubate at 60°C for 5~10 min. Mix by inverting the tube at frequent intervals.

2. Add 150 μl of Protein Precipitation Solution into the lysate. Mix well and incubate on ice for 5 min, then spin at top speed for 5 min at room temperature (25~28°C).

   ** The solution will become cloudy due to precipitation of detergent, proteins, polysaccharides and secondary metabolites.*

4. Transfer the supernatant to a RNA Spin Filter (with yellow ring) inserted in a clean 2 ml Collection Tube. Spin at top speed for 2 min.

   ** Most tissue clumps and cell debris can be removed by the spin filter, but a small amount will pass through and form a pellet in the collection tube. Do not disturb the pellet.*

5. Transfer the flow-through from the collection tube to a new 1.5 ml microcentrifuge tube, avoiding disturbing and pipetting the pellet.

6. Add 1.5 volumes of RNA Binding solution/Ethanol mixture to the lysate (flow-through), and mix well by vortexing or pipetting.

   ** For example: Add 750 μl of RNA Binding Solution/Ethanol mixture to 500 μl lysate.*

** A stringy precipitate may form after the addition of RNA Binding Solution/Ethanol mixture. This does not affect RNA isolation. Load the solution and precipitate into the RNA spin column in the next step.*

7. Load the lysate/Binding/ethanol mixture (<700 μl) to the RNA Spin Column inserted in a 2 ml collection tube, spin at top speed for 1 min and discard the flow-through.

   **If volume of* lysate/Binding/ethanol mixture *is greater than 700 μl, transfer any remaining* lysate/Binding/ethanol mixture *to the RNA Spin Column and repeat the above step.*

8. Transfer the RNA Spin Column into the original collection tube and add 500 μl RNA Wash Solution I, spin at top speed for 1 min and discard the flow-through.

9. **DNase I digestion**.
   For each isolation reaction, premix 80 μl of DNase I Incubation Buffer with 2 μl DNase I in a new sterile tube (Mix by flicking or inverting the tube, do not vortex!). Add 82 μl of the solution to the center of the RNA Spin Column membrane, and incubate at room temperature (25~28°C) for 15 min.
   ** If multiple extractions are performed, prepare a fresh mixture of DNase I Solution before use; do not store a premix of DNase I Solution.*

10. Add 500 μl of RNA Wash Solution I to the RNA Spin Column, spin at top speed for 1 min, and discard the flow-through.

11. Transfer the RNA Spin Column to the original collection tube. Add 600 μl of RNA Wash Solution II, spin at top speed for 1 min and then discard the flow-through. Repeat this step once more.

12. Place the RNA Spin Column into the original collection tube and

spin at top speed for 3 min to remove any residual ethanol. Transfer the RNA Spin Column to a clean 1.5 ml microcentrifuge tube.

** If centrifugation speed is lower than 12,000 xg or residual ethanol from RNA Wash Solution II must be removed completely, incubate the RNA Spin Column at 60~65°C in a drying oven for 5 min to evaporate all of the remaining ethanol.*

13. Add 30~50 μl of Nuclease-free water into the center of the RNA Spin Column membrane and let stand for 1 min. Centrifuge for 1 min at top speed and store the RNA sample at -70°C.

Troubleshooting:

Problem	Solutions
Column is clogged	
1. Too much tissue was used	It is essential to use the correct amount of starting material and make sure the plant tissue is sufficiently ground.
2. Centrifugation before adding ethanol was not performed	Centrifuge the lysate before adding ethanol. Pellets containing cell debris can clog the RNA spin column.
3. Starch-containing sample was heated at 60°C	Incubate starch-containing plant tissues (such as potato tuber, corn seed) in lysis solution at room temperature instead of at 60°C.
4. Plant tissue contains high level of lipids	Increase the centrifugation time by 5 min to pellet the cellular debris and avoid pipetting the free -floating lipid material.
Poor RNA yield	
1. Too much starting material.	Do not exceed 100 mg of tissue. For extremely difficult tissues, such as citrus leaf and old red maple leaves, start with 50 mg of plant tissue.
2. Starting sample contains small amount of total RNA	Yields will vary greatly between different types of plants.
3. Plant tissue was insufficiently ground	Grind plant tissue to a fine powder in liquid nitrogen. RNA yield is highly dependent on how well the tissue was ground prior to isolation
4. RNA still bound to the membrane	Repeat elution step, and incubate the column at room temperature for 10 min with Nuclease-free water before centrifugation.

Problem	Solutions
RNA is degraded	
1. RNase contamination	a) Use RNase-free solution, pipette tips, plastic -ware and glassware. b) Wipe laboratory environment with RNase/ DNase/EtBR Terminator (Cat# PT-R475). c) Change gloves frequently.
2. Improper handling of sample from harvest until lysis	a) If not processed immediately, snap-freeze tissue immediately after harvesting and store at -70°C or in liquid nitrogen. b) Samples must remain frozen until RNA Lysis/2-ME Solution is added. c) Tissues may be stored in RNAKeeper™ reagent
DNA contamination	
1. No DNase I treatment.	Be sure to add 2 µl DNase I (supplied with DNase I Incubation Buffer).
2. No incubation with RNA Wash Solution I before DNase I treatment.	Be sure to treat spin column with RNA Wash Solution I in step 2 before DNase I digestion.
RNA does not perform well in downstream experiments	
1. Ethanol carry-over	Be sure to dry the membrane by centrifugation at > 12,000 x *g* for 3 min. Following the centrifugation, incubate the RNA Spin Column at 60~65°C in a drying oven for 5 min to evaporate the remaining ethanol
2. Residual salt in eluate	Residual guanidine thiocyanate will also inhibit enzyme activities. Transfer the RNA Spin Column to a clean 1.5 ml microcentrifuge tube before adding RNA Wash Solution II.

Appendix I: RNA Isolation from highly viscous samples rich in polyphenols and polysaccharides Protocol

** This is a modified RNA Lysis Solution B protocol.*

Since the sample will be very viscous after lysis, which makes pipetting very difficult, lysis could take place directly in the Spin Filter inserted into a Collection Tube to reduce pipetting steps.

1. **Pre-chill the RNA Spin Filter (with yellow ring) inserted in a Collection Tube on ice,** then quickly weigh **no more than 30 mg** of fresh tissue, or frozen or RNA Keeper^{TM} stabilized tissue, and homogenize it thoroughly under liquid nitrogen. Then add the homogenate to the pre-chilled spin filter.
2. Add 600 µl **of Plant RNA Lysis Solution B/2-ME** into the pre-chilled spin filter and incubate at 60°C for 10 min in a heating block. Mix by inverting the spin filter column (not with collection tube) at frequent intervals during incubation.
3. Spin at top speed for 5 min and collect the flow-through solution from the collection tube into a new 1.5 ml microcentrifuge tube, avoiding pipetting the pellets.
4. *After passing through the RNA spin filter, the lysate will be much less viscous.*
5. *Some samples may clog the filter. In such cases, spin an additional 5 min or transfer the remaining lysate in the column to a new filter and spin again.*
6. Add 1/3 volume of **Protein Precipitation Solution** into the lysate and mix well. Incubate on ice for 5 min and centrifuge at top speed for 5 min at room temperature.
7. Carefully transfer the supernatant in to a new 1.5 ml microcentrifuge tube without disturbing and pipetting the pellets. Continue with step 6 of the **RNA Lysis Solution B** protocol on page 7.

Appendix II: Protocol for RNA Clean-Up or Genomic DNA Removal

* This kit can be used to clean up RNA or remove genomic DNA contamination from samples purified using different isolation methods.

1. Adjust RNA volume to 100 µl with Nuclease-free water. Add 350 µl of **RNA Lysis A/2-ME solution** and mix well.
2. Add 250 µl of 100% Ethanol and mix by pipetting. Continue to step 6 of the **RNA Lysis Solution A** protocol on page 4.

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