FastPure Plant Total RNA Isolation Kit (Polysaccharides&Polyphenolics-rich)

RC401

Instruction for Use

Version 23.1

Vazyme

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01/Product Description

This kit is suitable for the rapid extraction of total RNA from plant tissues, especially those rich in polysaccharides, polyphenols, or starch (e.g., cotton, mature rice, pine needles,populus, loquat, potato, banana, grape, apple, pear, China rose, buckwheat seeds, and *Arabidopsis thaliana* seeds), with extraction protocols provided for common plant tissues (e.g., *Arabidopsis thaliana* and tobacco). The kit is based on silica gel membrane purification technology without time-consuming alcohol precipitation and does not require phenol/chloroform, or any other toxic reagent during the extraction process. FastPure gDNA-Filter Columns II can effectively remove impurities, adsorbing and removing genomic DNA. FastPure RNA Columns IV can efficiently bind RNA, and with an optimized buffer the obtained total RNA has high purity. The isolated RNA has no proteins or other impurities contamination. It can be used for RT-PCR, Real-Time PCR, Microarrarys, Northern Blot, Dot Blot, Poly(A) screening, in vitro translation, RNase protection assay, molecular cloning and other downstream experiments.

02/Components

Components	RC401-01 (50 rxns)
Buffer PRL	30 ml
Buffer PRLPlus	25 ml
Buffer PRW1	40 ml
Buffer PRW2	12 ml
RNase-free ddH_2O	10 ml
FastPure gDNA-Filter Columns Ⅱ (each in a 2 ml Collection Tube)	50
FastPure RNA Columns Ⅳ (each in a 2 ml Collection Tube)	50
Collection Tubes 2 ml	50
RNase-free Collection Tubes 1.5 ml	50

Buffer PRL: Provide the environment required for plant tissues rich in polysaccharides and polyphenols.

Buffer PRLPlus: Provide the environment required for the separation of RNA and DNA (polysaccharide- and polyphenol-rich plants).

Provide the environment required for the lysis of plant tissues and separation of RNA and DNA (common plants).

Buffer PRW1: Remove proteins and DNA impurities.

Buffer PRW2: Remove residual salt ions.

RNase-free ddH_2O: Elute total RNA.

FastPure gDNA-Filter Columns II: Adsorb DNA and RNA, and remove lysis impurities.

FastPure RNA Columns IV: Specifically adsorb RNA.

Collection Tubes 2 ml: Collect filtrate.

RNase-free Collection Tubes 1.5 ml: Collect RNA.

03/Storage

Store at 15 ~ 25℃ and transport at room temperature.

04/Applications

50 - 100 mg of plant samples, such as pine needles and wheat leaves.

10 - 20 mg of seed samples, such as red bean seeds and wheat seeds.

05/Self-prepared Materials

β-Mercaptoethanol or 2 M DTT, absolute ethanol, RNase-free pipette tips, mortar, and water bath or thermal shaker.

▲Prepare DTT with RNase-free ddH_2O. Store at -20℃. Avoid repeated freezing and thawing.

06/Notes

For research use only. Not for use in diagnostic procedures.

1. **Polysaccharide- and polyphenol-rich plants:** Before the experiment, add 5% β-mercaptoethanol (or 2 M DTT) to Buffer PRL (add 25 μl of β-mercaptoethanol or 25 μl of 2 M DTT per 475 μl of Buffer PRL). It is recommended to prepare this lysis buffer fresh before use. Buffer PRL supplemented with β-mercaptoethanol or 2 M DTT can be stored at 2 ~ 8℃ for one month.
2. **Common plants:** Before the experiment, add β-mercaptoethanol to Buffer PRLPlus, following the same protocol for Buffer PRL.
   ▲Buffer PRL is not used for RNA isolation from common plants.
3. Please add 48 ml absolute ethanol to Buffer PRW2 according to the label, mark the bottle body and cap and mix well before use.
4. Check whether there is crystal precipitation in Buffer PRL and Buffer PRLPlus before use. If there is crystal precipitation, it can be placed at 65℃ to dissolve the precipitation, and then mixed thoroughly before use.
5. Sample processing: When you are exploring experiment conditions, it is recommended to start with a sample input amount of ≤50 mg. The input can be increased or decreased later based on the experiment results.
6. Buffer PRL, Buffer PRLPlus, and Buffer PRW1 contain irritants. Wear latex gloves when handling the buffers and avoid exposure to skin, eyes, or clothing. In case of skin or eye contact, flush with large amounts of water or saline, and seek medical attention if necessary.
7. Replenish liquid nitrogen during cryogenic grinding to prevent the sample from thawing.
8. This kit can remove 95% of DNA contamination from the reaction mix. In general, the purified RNA can be used for downstream experiments without DNase I treatment. If downstream experiments are sensitive to trace DNA, DNase I can be used to further remove DNA contamination.
9. Perform all steps at room temperature (15 ~ 25℃).

07/Mechanism & Workflow

Polysaccharide- and Polyphenol-rich Plants

Liquid Nitrogen Grinding: Grind the plant sample into powder in liquid nitrogen and take an appropriate amount of the sample for the experiment.

Tissue Lysis: Add 500 μl of Buffer PRL (pre-heated to 65℃ and supplemented with 5% β-mercaptoethanol or 5% 2 M DTT), vortex, and incubate in a 65℃ water bath for 5 min. Centrifuge at 12,000 rpm (13,400 × g) for 10 min and **take the supernatant**.

RNA and DNA Adsorption: Add 0.5 volumes of absolute ethanol to the supernatant and mix well. Transfer the mixture to a FastPure gDNA-Filter Columns II and centrifuge at 12,000 rpm (13,400 × g) for 2 min. **Discard the filtrate**.

→ gDNA+RNA

RNA Elution: Use a new Collection Tube. Add 500 μl of Buffer PRLPlus to the FastPure gDNA-Filter Columns II and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. **Collect the filtrate**.

→ gDNA
→ RNA

Adjustment of the Binding Environment: Add 0.5 volumes of absolute ethanol to the filtrate and mix well.

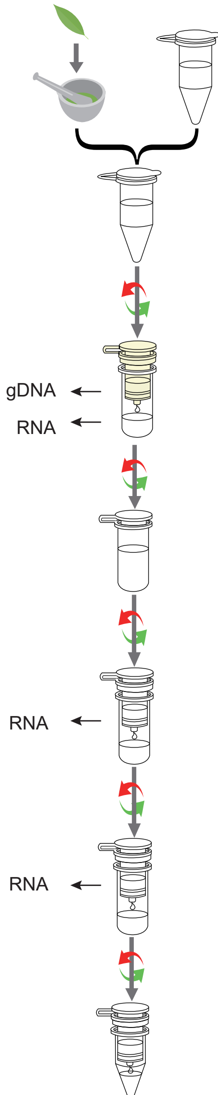
RNA Adsorption: Transfer all the mixture to a FastPure RNA Columns IV and centrifuge at 12,000 rpm (13,400 × g) for 2 min. **Discard the filtrate**.

→ RNA

Removal of Proteins and Other Impurities: Add 700 μl of Buffer PRW1 and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. **Discard the filtrate**.
Removal of Salt Ions: Add 500 μl of Buffer PRW2 (supplemented with absolute ethanol), centrifuge at 12,000 rpm (13,400 × g) for 30 sec, and **discard the filtrate.** Repeat once.
Ethanol Removal: Centrifuge the empty column at 12,000 rpm (13,400 × g) for 2 min.
RNA Elution: Add 30 - 100 μl of RNase-free ddH_2O and centrifuge at 12,000 rpm (13,400 × g) for 1 min.

→ RNA

Common Plants



Liquid Nitrogen Grinding: Grind the plant sample into powder in liquid nitrogen and take an appropriate amount of the sample for the experiment.

Tissue Lysis: Add 500 μl of Buffer PRLPlus (supplemented with 5% β-mercaptoethanol or 5% 2 M DTT), vortex, and centrifuge at 12,000 rpm (13,400 × g) for 5 min. **Take the supernatant**.

gDNA Removal: Transfer the supernatant to a FastPure gDNA-Filter Columns II and centrifuge at 12,000 rpm (13,400 × g) for 2 min. **Collect the filtrate**.

Adjustment of the Binding Environment: Add 0.5 volumes of absolute ethanol to the filtrate and mix well.

RNA Adsorption: Transfer all the mixture to a FastPure RNA Columns IV and centrifuge at 12,000 rpm (13,400 × g) for 2 min. **Discard the filtrate**.

Removal of Proteins and Other Impurities: Add 700 μl of Buffer PRW1 and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. **Discard the filtrate**.
Removal of Salt Ions: Add 500 μl of Buffer PRW2 (supplemented with absolute ethanol), centrifuge at 12,000 rpm (13,400 × g) for 30 sec, and **discard the filtrate**. Repeat once.
Ethanol Removal: Centrifuge the empty column at 12,000 rpm (13,400 × g) for 2 min.
RNA Elution: Add 30 - 100 μl of RNase-free ddH$_2$O and centrifuge at 12,000 rpm (13,400 × g) for 1 min.

08/Experiment Process

08-1/Polysaccharide- and Polyphenol-rich Plants

This protocol is suitable for the extraction of RNA from polysaccharide- and polyphenol-rich plant samples, including woody perennials (e.g., pine, ginkgo, and cotton), polyphenol-rich pulp (e.g., grape and banana), seeds (e.g., wheat, red bean, and peanut seeds), and fungi (e.g., shiitake and oyster mushroom). If you are unsure about the type of plant used, it is recommended to start with the protocol for common plants.

Sample Processing

Liquid Nitrogen Grinding:

Quickly grind the plant sample to powder in liquid nitrogen. Store at -85 ~ -65℃ if the ground sample is not used immediately for the next step.

RNA Extraction

Perform the following steps in an RNase-free fume hood.

1. Weigh an appropriate amount of ground plant sample, and add 500 μl of Buffer PRL (pre-heated to 65℃ and supplemented with 5% β-mercaptoethanol or 5% 2 M DTT before use). Immediately vortex vigorously for 30 - 60 sec to thoroughly lyse the sample and reduce the viscosity so as to increase the yield.
   ▲ Use 20 mg of starting material for samples with low water content, such as mature leaves, seeds, and starchy tubers. The input amount can be increased for samples with high water content, such as strawberries and watermelons.
   ▲ Before the experiment, add 5% β-mercaptoethanol or 5% 2 M DTT to Buffer PRL (25 μl of β-mercaptoethanol or 25 μl of 2 M DTT per 475 μl of Buffer PRL), mix by inversion, and pre-heat in a 65℃ water bath. Scale up the volume proportionally if extraction is performed for multiple samples.
   ▲ Store at -85 ~ -65℃ if the ground sample is not used immediately for the next step.
2. Incubate the lysate in the 65℃ water bath for 5 min, inverting the tube 1 - 2 times to facilitate lysis. Centrifuge at 12,000 rpm (13,400 × g) for 10 min.
3. Transfer the supernatant to a new 1.5 ml RNase-free centrifuge tube, add 0.5 volumes of absolute ethanol, and immediately mix well by pipetting up and down.
   ▲ Precipitates may form in this process. Proceed directly to Step 4 after mixing.
   ▲ Calculate the volume of absolute ethanol added based on the actual volume of the supernatant. For example: add 200 μl of absolute ethanol to 400 μl of supernatant.
4. Transfer the above mixture to a FastPure gDNA-Filter Columns II (FastPure gDNA-Filter Columns II had been put into the collection tube). Centrifuge at 12,000 rpm (13,400 × g) for 2 min, and **discard the filtrate**.
   ▲ The volume of the adsorption column is 700 μl. If the volume of the mixture exceeds 700 μl, centrifuge successive aliquots in the same FastPure gDNA-Filter Columns II. Discard the filtrate after each centrifugation.
   ▲ Ensure that all liquid has passed through after centrifugation with no residual liquid on the membrane. Increase centrifugation speed and time if necessary.

5. Place the FastPure gDNA-Filter Columns II into a new 2 ml Collection Tube (provided with the kit). Add 500 μl of Buffer PRLPlus, centrifuge at 12,000 rpm (13,400 × g) for 30 sec, and **collect the filtrate**.
6. Add 0.5 volumes of absolute ethanol to the filtrate and immediately mix well by pipetting up and down.
   ▲ Precipitates may form in this process. Proceed directly to Step 7 after mixing.
7. Transfer the above mixture to a FastPure RNA Columns IV (FastPure RNA Columns IV had been put into the collection tube). Centrifuge at 12,000 rpm (13,400 × g) for 2 min, and **discard the filtrate**.
8. Add 700 μl of Buffer PRW1 to the FastPure RNA Columns IV, incubate at room temperature for 1 min, and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. **Discard the filtrate**.
9. Add 500 μl of Buffer PRW2 (check whether 48 ml of absolute ethanol has been added before use) to the FastPure RNA Columns IV and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. **Discard the filtrate**.
10. Repeat Step 9.
11. Place the FastPure RNA Columns IV back into the Collection Tube. Centrifuge at 12,000 rpm (13,400 × g) for 2 min to remove the residual Buffer PRW2 from the FastPure RNA Columns IV.
    ▲ After centrifuging the empty column, air dry the column in a fume hood for 2 - 5 min for the residual ethanol to fully evaporate.
12. Transfer the FastPure RNA Columns IV into a new 1.5 ml RNase-free Collection Tube, and add 30 - 100 μl of RNase-free ddH_2O to the center of the membrane without touching the column. Incubate at room temperature for 2 min and centrifuge at 12,000 rpm (13,400 × g) for 1 min.
    ▲ The following steps can help increase product RNA concentration:
    Pre-heat RNase-free ddH_2O at 65℃.
    Incubate at room temperature for 5 min.
    Load the eluent from the first elution back onto the adsorption column for a second elution.
13. The extracted RNA can be used directly for downstream experiments or stored at -85 ~ -65℃.

08-2/Common Plants

This protocol is suitable for the extraction of RNA from non-polysaccharide- or polyphenol-rich plant samples, including the young leaves of annual and biennial plants (e.g., *Arabidopsis thaliana*, wheat, rice, and tobacco) and the pulp of most fruits (e.g., peach, pear, apple, and tomato).

Sample Processing

Liquid Nitrogen Grinding:

Quickly grind the plant sample to powder in liquid nitrogen. Store at -85 ~ -65℃ if the ground sample is not used immediately for the next step.

RNA Extraction

Perform the following steps in an RNase-free fume hood.

1. Weigh an appropriate amount of ground plant sample, and add 500 μl of Buffer PRLPlus (supplemented with 5% β-mercaptoethanol or 5% 2 M DTT before use). Immediately vortex vigorously for 30 - 60 sec to thoroughly lyse the sample and centrifuge at 12,000 rpm (13,400 × g) for 5 min.
2. Transfer the supernatant to a FastPure gDNA-Filter Columns II (FastPure gDNA-Filter Columns II had been put into the collection tube). Centrifuge at 12,000 rpm (13,400 × g) for 2 min, and **collect the filtrate**.
3. Add 0.5 volumes of absolute ethanol to the filtrate and immediately mix well by pipetting up and down.
4. Transfer the above mixture to a FastPure RNA Columns IV (FastPure RNA Columns IV had been put into the collection tube). Centrifuge at 12,000 rpm (13,400 × g) for 2 min, and **discard the filtrate**.
5. Add 700 μl of Buffer PRW1 to the FastPure RNA Columns IV, incubate at room temperature for 1 min, and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. **Discard the filtrate**.
6. Add 500 μl of Buffer PRW2 (check whether 48 ml of absolute ethanol has been added before use) to the FastPure RNA Columns IV and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. **Discard the filtrate**.
7. Repeat Step 6.
8. Place the FastPure RNA Columns IV back into the Collection Tube. Centrifuge at 12,000 rpm (13,400 × g) for 2 min to remove the residual Buffer PRW2 from the FastPure RNA Columns IV.

   ▲ After centrifuging the empty column, air dry the column in a fume hood for 2 - 5 min for the residual ethanol to fully evaporate.
9. Transfer the FastPure RNA Columns IV into a new 1.5 ml RNase-free Collection Tube, and add 30 - 100 μl of RNase-free ddH_2O to the center of the membrane without touching the column. Incubate at room temperature for 2 min and centrifuge at 12,000 rpm (13,400 × g) for 1 min.

   ▲ The following steps can help increase product RNA concentration:
   Pre-heat RNase-free ddH_2O at 65℃.
   Incubate at room temperature for 5 min.
   Load the eluent from the first elution back onto the adsorption column for a second elution.
10. The extracted RNA can be used directly for downstream experiments or stored at -85 ~ -65℃.

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09/FAQ & Troubleshooting

FAQ	Reasons	Solutions
Clogged FastPure gDNA-Filter Columns II	1. Too much sample	Reduce the sample input amount.
	2. Incomplete grinding of sample	Grind the sample as thoroughly as possible. Increase the volume of lysis buffer and extend centrifugation time if necessary. Do not disturb the precipitate when taking the supernatant.
Low or no recovery of RNA	1. Unsuitable protocol	Use the other protocol.
	2. Insufficient sample lysis	Ensure thorough grinding of the sample in liquid nitrogen, and quickly transfer the ground sample into the lysis buffer prepared in advance.
	3. Too little or too much input	Follow the Instructions for Use for the sample input amount.
	4. Incomplete elution	Preheat RNase-free ddH_2O at 65℃. Add the RNase-free ddH_2O to the center of the membrane. Reduce the elution volume appropriately. Extend the elution time, or perform a second elution.
RNA degradation	1. Improper or prolonged sample storage	Use a fresh sample or a sample flash frozen in liquid nitrogen and stored at -85 ~ -65℃.
	2. Repeated freezing and thawing of the sample	Store samples in aliquots to avoid degradation caused by repeated freezing and thawing.
	3. Electrophoresis issues	Before running the gel, soak the electrophoresis tank in 3% hydrogen peroxide for 20 min, and then rinse with RNase-free ddH_2O. Prepare the electrophoresis buffer with RNase-free ddH_2O.
	4. RNase contamination	Ensure that the pipette tips and centrifuge tubes used are RNase-free.
Inhibition of downstream assays or low purity	1. Residual salt ions	Ensure the column is rinsed twice with Buffer PRW2. Add Buffer PRW2 along the wall of the adsorption column, or cap the column and invert 2 - 3 times after adding Buffer PRW2 to fully rinse the salt attached to the column wall.
	2. Residual ethanol	After centrifuging the empty column, allow it to stand at room temperature for 5 min to fully remove residual ethanol.
Genomic DNA contamination	1. Too much sample	Reduce the sample input amount.
		Use a kit that contains a genome removal module for reverse transcription, such as HiScript III RT SuperMix for qPCR (+gDNA wiper) (Vazyme #R323).
		Use intron-spanning primers to avoid amplification of genomic DNA.
		Use on-column DNase I treatment to remove DNA contamination.

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