FastPure Universal Plant Total RNA Isolation Kit

RC411

Vazyme

Instruction for Use

Version 22.1

Vazyme

Contents

01/Product Description

This kit is suitable for rapid extraction of total RNA from plant tissues. The kit contains two sets of solution systems, which can solve the RNA extraction of various simple plant tissues (wheat, rice, corn, arabidopsis, tobacco, rape, etc.), polysaccharides and polyphenolics-rich plant tissues (cotton leaves, soybean leaves, pine needles, ginkgo leaves, fig leaves, gardenia leaves, wheat seeds, corn seeds, red bean seeds, potatoes, sweet potatoes, soybean seeds, sesame seeds, peanut seeds, rape seeds, etc.), fruit flesh (watermelons, apples, peaches, pears, bananas, mangos, etc.), and fungi (*Lentinus, Tricholoma gambosum, Pleurotus ostreatus, Neurospora crassa*, etc.). The kit is based on silica gel membrane purification technology and does not require phenol/chloroform, β-mercaptoethanol, or any other toxic reagent and time-consuming alcohol precipitation during the extraction process. It takes only 11 min to extract RNA. FastPure gDNA-Filter Columns III can effectively remove impurities and gDNA. FastPure RNA Columns V can efficiently bind RNA, and with an optimized buffer the obtained total RNA has high purity. The isolated RNA has little gDNA residues and no proteins or other impurities contamination. It can be used for RT-PCR, Real-Time PCR, RNA library construction, microarray analysis, Northern Blot, Dot Blot, molecular cloning and other downstream experiments.

02/Components

Components	RC411-01 (50 rxns)
Buffer EL	40 ml
Buffer PSL	40 ml
Buffer RWA	40 ml
Buffer RWB	12 ml
RNase-free ddH_2O	10 ml
FastPure gDNA-Filter Columns III (each in a 2 ml Collection Tube)	50
FastPure RNA Columns V (each in a 2 ml Collection Tube)	50
RNase-free Collection Tubes 1.5 ml	50

Buffer EL: Provide the environment required for simple plant tissues lysis.
Buffer PSL: Provide the environment required for polysaccharides and polyphenolics-rich plant tissues lysis.
Buffer RWA: Remove impurities such as proteins and DNA.
Buffer RWB: Remove salt ion residues.
RNase-free ddH_2O: Elute total RNA.
FastPure gDNA-Filter Columns III: Adsorb DNA and remove impurities in the lysate.
FastPure RNA Columns V: Adsorb RNA specifically.
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

Leaves: 50 - 100 mg

Polysaccharide tubers, tuberous roots, seeds: 20 - 50 mg

Fruit flesh: 100 - 200 mg

Fungi: 20 - 100 mg

05/Self-prepared Materials

Absolute ethanol, RNase-free pipette tips, mortars, etc.

06/Notes

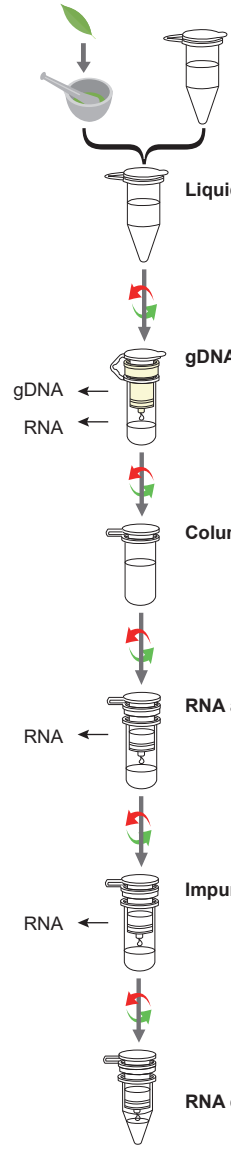
For research use only. Not for use in diagnostic procedures.

1. Please add 48 ml absolute ethanol to Buffer RWB according to the label, mark the bottle body and cap, and mix well before use.
2. Check whether there is crystal precipitation in Buffer EL and Buffer PSL before use. If there is crystal precipitation, heat the Buffer in a 65℃ water bath to dissolve the precipitation, then mix thoroughly before use.
3. Sample processing: Perform the experiment within the rage of recommended input amount, and increase or decrease the subsequent input amount according to the experimental situation. Do not exceed the recommended input amount, because excessive input amount will lead to gDNA residue or low yield.
4. When using fresh samples, if extraction is not performed immediately, the samples should be placed in liquid nitrogen immediately and stored at -85 ~ -65℃ after quick-freezing. And avoid repeated freezing and thawing. Sample collection and storage should be carried out as quickly as possible to prevent RNA degradation.
5. When grinding using liquid nitrogen, replenish liquid nitrogen in time to prevent the samples from thawing.
6. When the sample is taken out of the liquid nitrogen, it needs to be added to the lysate immediately and mixed evenly. Do not leave the sample at room temperature alone. The melting of the sample or uneven mixing of the sample and the lysate will lead to RNA degradation.
7. Plant tissues with high starch contents (such as potatoes, sweet potatoes, seeds, etc.) react with lysis buffer at room temperature will produce gelatinous substances, and the longer the contact time, the more gelatinous substances produced. Therefore, samples should be centrifuged as soon as possible after lysis, and transfer the supernatant to FastPure gDNA-Filter Columns III. When taking the supernatant, do not absorb the gelatinous substances to avoid clogging FastPure gDNA-Filter Columns III.

   ▲ If higher yield RNA is required for such samples, FastPure Plant Total RNA Isolation Kit (Polysaccharides & Polyphenolics-rich) (Vazyme #RC401) is recommended.

8. Buffer EL, Buffer PSL, and Buffer RWA contain irritating compounds, so wear disposable latex gloves when operating to avoid contact with skin, eyes and clothes. In case of contact with skin or eyes, rinse with plenty of water or normal saline, and seek medical treatment if necessary.
9. Change gloves frequently and use RNase-free plastics consumables and pipette tips to avoid RNase contamination.
10. This kit can remove most of genomic DNA. The RNA obtained can be directly used for downstream experiments without DNase I treatment. The nucleic acid content of different samples varies greatly. If the downstream experiments are very sensitive to trace amounts of DNA, DNase I can be used to further remove genomic DNA thoroughly.
11. All procedures must be carried out at room temperature (15 ~ 25℃).

07/Mechanism & Workflow



Liquid nitrogen grinding: Quickly grind plant samples into powder in liquid nitrogen, and take appropriate samples for the experiment. Refer to **08-2/Lysis Buffer Selection** to select lysis buffer.

Liquid nitrogen grinding: Add 600 µl Buffer EL (simple plants) or 600 µl Buffer PSL (polysaccharides and polyphenolics-rich plants). Mix by vortexing, and immediately centrifuge at 12,000 rpm (13,400 × g) for 5 min. **Pipette the supernatant.**

gDNA removal: Transfer the supernatant to FastPure gDNA-Filter Columns III and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. **Collect the filtrate**.

Column environment adjustment: Add absolute ethanol (0.5 × the volume of filtrate) to the filtrate and mix thoroughly.

RNA adsorption: Transfer the mixture to FastPure RNA Columns V and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. **Discard the filtrate.**

Impurity removal: Add 700 µl Buffer RWA and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. **Discard the filtrate.**
Add 500 µl Buffer RWB (with absolute ethanol added) and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. **Discard the filtrate.** Repeat this step.
Centrifuge the empty column at 12,000 rpm (13,400 × g) for 2 min.

RNA elution: Add 30 - 100 µl RNase-free ddH_2O and centrifuge at 12,000 rpm (13,400 × g) for 1 min.

08/Experiment Process

08-1/Sample Processing

Quickly transfer fresh or frozen plant tissues at -85 ~ -65℃ to a mortar pre-cooled with liquid nitrogen. Grind the tissues, and continuously add liquid nitrogen to keep the tissues at a low temperature until they are ground into powder (No visible particles. Insufficient grinding will affect the RNA extraction efficiency and quality).

08-2/Lysis Buffer Selection

Sample types	Recommended input amount	Material examples	Recommended lysis buffer	
			Buffer EL	Buffer PSL
Simple plant tissues (tender leaves, stems, roots)	50 - 100 mg	wheat, rice, corn, arabidopsis, tobacco, rape, etc	√	
polysaccharides and polyphenolics-rich plant leaves		cotton leaves, soybean leaves, pine needles, ginkgo leaves, fig leaves, gardenia leaves, etc.		√
Plant tissues with high starch contents	20 - 50 mg	wheat seeds, corn seeds, red bean seeds, potatoes, sweet potatoes, etc.		√
Plant tissues with high oil contents		soybean seeds, sesame seeds, peanut seeds, rapeseeds, etc.	√	√
fruit flesh	100 - 200 mg	watermelons, apples, peaches, pears, bananas, mangos, etc.	√	
fungi	20 - 100 mg	Lentinus, Tricholoma gambosum, Pleurotus ostreatus, Neurospora crassa, etc.		√

▲If the plant types are not sure, it is recommended to try Buffer PSL first.

08-3/RNA Extraction

1. Determine sample input amount and appropriate lysis buffer according to 08-2/Lysis Buffer Selection. Take the correct amount of plant tissues ground by liquid nitrogen and immediately add 600 µl Buffer EL or 600 µl Buffer PSL. Mix the samples and lysis buffer thoroughly by vortexing vigorously for 30 sec. Centrifuge at 12,000 rpm (13,400 × g) for 5 min, then perform subsequent operations immediately.

   ▲ After the samples are taken out from liquid nitrogen, immediately add it to the lysis buffer and mix well. Do not place samples at room temperature. Sample thawing or uneven mixing of the sample and lysis buffer will lead to RNA degradation.

   ▲ Plant tissues with high starch contents (such as potatoes, sweet potatoes, seeds, etc.) react with lysis buffer at room temperature to produce gelatinous substances, and the longer the contact time, the more gelatinous substances produced. Therefore, samples should be centrifuged as soon as possible after lysis, and transfer the supernatant to FastPure gDNA-Filter Columns III. When taking the supernatant, do not absorb the gelatinous substances to avoid clogging FastPure gDNA-Filter Columns III.

   ▲ A small amount of floating matter in the supernatant after centrifugation is normal and the subsequent experiments can be carried out directly.

2. Transfer about 500 μl supernatant to FastPure gDNA-Filter Columns III (FastPure gDNA-Filter Columns III had been put into the collection tube) and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the FastPure gDNA-Filter Columns III and collect the filtrate.
   ▲ The pipetted volume of supernatant can be adjusted according to the actual situation.
   ▲ FastPure gDNA-Filter Columns III has a good filtering effect on impurities, so absorbing a small number of sample fragments will not affect subsequent experiments.
3. Add 0.5 times the filtrate volume of absolute ethanol (about 250 μl and it can be adjusted according to the actual situation) to the collection tube and mix well by vortexing for 15 sec.
   ▲ It is normal to appear turbidity or flocculent precipitate after adding ethanol, and the mixture (including flocculent matter) can be continued for subsequent operations.
4. Transfer the mixture from step 3 to FastPure RNA Columns V (FastPure RNA Columns V had been put into the collection tube) and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. **Discard the filtrate**.
   ▲ The adsorption column volume is 750 μl. If the volume of the mixture exceeds 750 μl, please transfer the solution in several times.
5. Add 700 μl Buffer RWA to the FastPure RNA Columns V and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. **Discard the filtrate**.
6. Add 500 μl Buffer RWB (check if absolute ethanol has been added in advance!) to the FastPure RNA Columns V 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 V back into the collection tube and centrifuge at 12,000 rpm (13,400 × g) for 2 min.
9. Transfer the FastPure RNA Columns V to new RNase-free Collection Tubes 1.5 ml. Add 30 - 100 μl RNase-free ddH_2O to the center of the adsorption column without touching the membrane and centrifuge at 12,000 rpm (13,400 × g) for 1 min.
   ▲ The elution volume is recommended to be no less than 30 μl, because too small volume will affect the recovery efficiency of nucleic acid.
   ▲ The following steps can help increase the concentration of RNA product:
   RNase-free ddH_2O could be preheated at 65℃ in advance.
   After adding the RNase-free ddH_2O, incubate at room temperature for 5 min.
   The first elution product could be added to the adsorption column for secondary elution.
10. The extracted RNA can be directly used for downstream experiments or stored at -85 ~ -65℃.

09/FAQ & Troubleshooting

FAQ	Reasons	Solutions
Clogged Column	1. Excessive impurities of sample	Reduce the input amount of sample or increase the volume of lysis buffer.
	2. Insufficient lysis of sample	Grind as thoroughly as possible. If necessary, increase the volume of lysis buffer and prolong the centrifugation time. When taking the supernatant, try not to absorb a large amount of precipitate, a small amount of precipitate will not affect subsequent experiments.
	3. Excessive input amount of sample	The input amount of sample is recommended to follow the instruction.
Low or no recovery of RNA	1. Improper storage of sample	The tissues removed from plant should be immediately quick-frozen in liquid nitrogen, and then stored at -85 ~ -65℃. Avoid repeated freezing and thawing of sample.
	2. Insufficient lysis of sample	When grinding with liquid nitrogen, ensure that the grinding is sufficient. Quickly add lysis buffer and mix well. Failure to mix the sample and lysis buffer well in time may result in RNA degradation or low yield.
	3. Too high or too low input amount of sample	The input amount of sample is recommended to follow the instructions.
	4. Insufficient elution	The elution volume is no less than 30 μl. RNase-free ddH_2O could be preheated at 65℃ in advance. After adding the RNase-free ddH_2O to the center of membrane, incubate the tube at room temperature for 5 min or perform secondary elution.
RNA degradation	1. Improper storage of sample or too long storage time	Use fresh samples or samples at -85 ~ -65℃ that have never been thawed.
	2. Repeated freezing and thawing of sample	Avoid freezing and thawing the sample repeatedly and store it in aliquots.
	3. Reasons of electrophoresis	Before the electrophoresis, soak the electrophoresis tank in 3% hydrogen peroxide for 20 min and rinse it with RNase-free ddH_2O. Ensure that the electrophoresis buffer was prepared using RNase-free ddH_2O and that the extraction was performed in an RNase-free environment.
	4. RNase contamination	Ensure that the pipette tips and centrifuge tubes used in the extraction process are RNase-free.
Inhibition of downstream reaction or low purity	1. Salt ion residues	Ensure that wash with Buffer RWB is performed twice. Additionally, add Buffer RWB to the sides of the adsorption column or close the lid of the column and invert 2 to 3 times after adding Buffer RWB, which can help to completely wash away the salts on the sides of the column.
	2. Ethanol residues	After centrifuging the empty column incubate at room temperature for 5 min to remove the ethanol residues to the greatest extent.

gDNA contamination	1. Excessive input amount of sample	Reduce the input amount of the sample.
		Remove DNA contamination by on-column DNase digestion. Selecte the reverse transcription reagents containing genome removal modules for reverse transcription. It is recommended to use HiScript III RT SuperMix for qPCR (+gDNA wiper) (Vazyme #R323).
		Use the primers that span introns to avoid the involvement of genomic DNA templates in the amplification reaction.

Nanjing Vazyme Biotech Co.,Ltd.
Tel: +86 25-83772625
Email: info.biotech@vazyme.com
Web: www.vazyme.com
Loc: Red Maple Hi-tech Industry Park, Nanjing, PRC
Follow Us