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Plant Total RNA Extraction Kit Instructions

Composition

Plant Total RNA Extraction Kit Cat. No.	5 Preps 5101005	50 Preps 5101050
Filter Columns	5	50
Spin Columns	5	50
Buffer RLT	4 ml	32 ml
Buffer RLC	4 ml	32 ml
Buffer WA	1.9 ml	12 ml
Buffer WBR	1.5 ml	10 ml
RNase-free Water	1.5 ml	2 ml×3
Instructions	1	1

Storage

If the kit is stored at room temperature (0~30°C), it can keep the performance unchanged for 2 years, and if the product is stored at 2~8°C, the validity period can be extended to more than 2 years.

Technical Support

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Introduction

The kit includes two lysis buffer systems, which can solve the RNA extraction of various simple plant tissues, polysaccharide polyphenol-rich plant tissues, fruit pulp, fungi, etc. There is no need to use toxic reagents such as phenol, chloroform, β-merlidtoethanol, etc., and there is no need for time-consuming alcohol precipitation during the kit extraction process, and total RNA can be quickly extracted. The plant tissue lysate mixed with ethanol then added to the Spin Column, the RNA binds to the Spin Column, and the solubilized protein and PCR inhibitors are filtered out. After washing with two wash buffers, the RNA is eluted with RNase-free water, which can be used for various molecular biology experiments such as RT-PCR, Northern blot, Dot blot, mRNA isolation, etc.

Equipment And Reagents to Be Supplied by Users

1. Absolute ethanol and 70% ethanol.
2. RNase-free 1.5 ml centrifuge tubes.
3. Pipettes and tips (RNase-free pipette tips with filters are recommended to avoid RNase contamination).
4. Disposable gloves and protective equipment and tissues.
5. Microcentrifuge (s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
6. Vortexer
7. RNase-free use labs.

Preparation before Use

1. If the centrifuge has refrigeration function, set the temperature to 25°C.
2. Add absolute ethanol to Buffer WA and Buffer WBR according to the instructions on the label of the reagent bottle and tick the box on the label to mark "Ethanol added".
3. Because saliva and skin contain RNases, latex gloves and masks are required during the whole process of RNA extraction.
4. If DNA needs to be completely removed in subsequent experiments, please add a DNase I digestion step, and the DNase I Column Digestion Kit (Simgen, Cat. No.8010050) needs to be purchased by yourself.

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Lysis buffer Selection Guide

Sample type	Dosage	Sample examples	Lysis buffer	
			Buffer RLT	Buffer RLC
Simple plant tissue (young leaves, stems, roots)	50-100 mg	Wheat, rice, corn, Arabidopsis, tobacco, rape, etc.	√	
Polysaccharide polyphenol plant leaves	50-100 mg	Cotton leaves, soybean leaves, pine needles, ginkgo biloba, fig leaves, gardenia leaves, etc.		√
Plant tissues with a high starch content	20-50 mg	Wheat seeds, corn seeds, red bean seeds, potatoes, sweet potatoes, etc.		√
Vegetable tissues with a high fat content	20-50 mg	Soybean seeds, sesame seeds, peanut seeds, rapeseed, etc.	√	√
Fruit pulp	100-200 mg	Watermelon, apple, peach, pear, banana, mango, etc.	√	
fungi	20-100 mg	Shiitake mushrooms, mouth mushrooms, oyster mushrooms, crude vampires, etc.		√

* If the type of sample is not known, Buffer RLC may be preferred.

Protocol

1. **According to the "Lysis buffer Selection Guide", determine the sample dosage and applicable lysis buffer, grind the tissue to powder form with liquid nitrogen, weigh the appropriate amount of tissue with a 1.5 ml centrifuge tube pre-cooled with liquid nitrogen, add 600 μl Buffer RLT or 600 μl Buffer RLC, vortex and shake until the tissue is completely lysed, and centrifuge at 13,000 rpm for 2 min.**

* When grinding the tissue, liquid nitrogen should be added in time to avoid the tissue thawing, so as to avoid the degradation of RNA due to the reactivation of endogenous RNase.
* Do not use too much tissue, as this may cause clogging of the filter column and contamination of the purified RNA with genomic DNA.
* Fresh shoots contain high nucleic acid content, and genomic DNA may remain a lot, so DNase I digestion is recommended.
* Buffer RLT、Buffer RLC are corrosive, please wear protective equipment for operation.

2. **Transfer all the supernatant from step 1 into the Filter Column, close the lid, and centrifuge at 13,000 rpm for 2 min.**

* A small amount of sample debris in the supernatant can also be removed by the filter column without affecting subsequent experiments.
* If the lysate does not fully pass through the filter column, the nucleic acid content in the tissue is too high. At this time, 300 μl filtrate should be transferred to a clean 1.5 ml centrifuge tube, then 300 μl 70% ethanol should be added to the 1.5 ml centrifuge tube and piped 6-8 times with a pipette tip to mix well, then the whole mixture should be transferred to the Spin Column. close the lid, centrifuge at 13,000 rpm for 1 min. Discard the filter column and the filtrate, then proceed directly to step 5.

3. **Discard the Filter Column, add 600 μl 70% ethanol to the filtrate and directly pipette 6~8 times to mix evenly, transfer 600 μl mixture to a Spin Column, close the lid, and centrifuge at 13000 rpm for 1 min.**

* If there is a precipitate after mixing with 70% ethanol, please add the precipitate to the Spin Column.

4. **Discard the filtrate, place the Spin Column back into the 2 ml collection tube, transfer the remaining mixture to the Spin Column, and centrifuge at 13,000 rpm for 1 min.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhered to the mouth of the collection tube, you can slap the 2 ml collection tube upside down on a paper towel once.

5. **Discard the filtrate, add 500 μl Buffer WA to the Spin Column, close the lid, and centrifuge at 13,000 rpm for 1 min.**

* Ensure that absolute ethanol has been added to Buffer WA.

6. **Discard the filtrate, add 600 μl Buffer WBR to the Spin Column, close the lid, and centrifuge at 13,000 rpm for 1 min.**

* Ensure that absolute ethanol has been added to Buffer WBR.

7. **Discard the filtrate, place the Spin Column back into the 2 ml collection tube, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.
* Do not omit this step, otherwise the subsequent RT-PCR effect may be affected due to the ethanol mixed in the purified nucleic acid.

8. **Discard the 2 ml collection tube, place the Spin Column in a clean RNase-free 1.5 ml centrifuge tube, add 50~100 μl RNase-free Water to the Spin Column center, close the lid, incubate at room temperature for 1 min, and centrifuge at 13000 rpm for 1 min.**

* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm for 1 min to avoid damage to the centrifuge due to the detachment of the lid of the 1.5 ml centrifuge tube.

9. **Discard the Spin Column, the eluted RNA can be immediately used in various molecular biology experiments or stored below -70°C for later use.**