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Polysaccharides & Polyphenolics-rich Plant Total RNA Kit Instructions

Composition

Polysaccharides & Polyphenolics-Rich Plant Total RNA Kit Cat. No.	5 Preps 5103005	50 Preps 5103050
Filter Columns	5	50
Spin Columns	5	50
β-mercaptoethanol	50 µl	500 µl
Buffer RCT	4 ml	32 ml
Buffer EX	4 ml	32 ml
Buffer K	2 ml	20 ml
Buffer WA	1.9 ml	12 ml
Buffer WBR	1.5 ml	10 ml
RNase-free Water	1.5 ml	2 ml×3
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Storage

If the kit is stored at room temperature (0~30℃), it can keep the performance unchanged for 2 years, and if stored at 2~8℃, the validity period can be extended to more than2 years.

Technical Support

R&D Department, Hangzhou Simgen Biotechnology Co., Ltd. E-mail: technical@simgen.cn, Tel: 400-0099-857.

Introduction

This product is suitable for extract total RNA from 50 -200 mg plants. The supernatant containing RNA was extracted from plant tissues by Buffer RCT and Buffer EX. Ethanol was added to the supernatant and the RNA in mixture was bound to the Spin Column, and dissolved proteins and PCR inhibitors were filtered out. RNA was washed by two wash buffers and eluted with RNase-free Water, which could be used for various molecular biology experiments such as RT-PCR, Northern blot, Dot blot, mRNA separation.

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 tubes).
6. Vortexer
7. RNase-free use labs.

Preparation Before Use

1. If there is a precipitate in Buffer RCT, please incubate at 60℃ to dissolve it before use.
2. If the centrifuge has refrigeration function, set the temperature to 25℃.
3. Add 10 µl β-mercaptoethanol to per 1 ml Buffer RCT and mix well, within one month did not affect the experimental results.
4. 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.
5. Because saliva and skin contain RNases, latex gloves and masks are required during the whole process of RNA extraction.
6. 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.

Protocol

1. **Weigh 200~500 mg of plant tissue in a mortar, grind the tissue to powder form with liquid nitrogen, and then weigh 50~200 mg of the tissue with a 1.5 ml centrifuge tube pre-cooled with liquid nitrogen.**

* 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.
* Only samples with low RNA content (e.g., potato tubers, watermelon pulps, etc.) should be recommended to use 200 mg of tissue, and for samples with high RNA content such as young leaves and shoots, the dosage should be controlled within 100 mg, otherwise it may lead to blockage of the filter column or Spin Column. Fresh shoots contain high nucleic acid and may have a high amount of genomic DNA residue, so DNase I digestion maybe recommended.

2. **Add 600 µl Buffer RCT with β-mercaptoethanol, vortex until all the tissue lysed.**
3. **Add 600 µl Buffer EX, mix vigorously, and centrifuge at 12,000 rpm for 5 min.**
4. **Carefully transfer 350 µl supernatant to a clean 1.5 ml centrifuge tube.**

* Be careful not to bring in the protein precipitate in middle phase.

5. **Add 350 µl Buffer K to the supernatant and pipette 6-8 times directly with a pipette tip to mix well, transfer the mixture to a Filter Column, close the lid, and centrifuge at 13,000 rpm for 1 min.**

* Do not omit this step, as this may cause clogging of the Spin Column in subsequent steps.

6. **Discard the Filter Column, add 700 µl 70% ethanol to the filtrate and directly pipette 6-8 times to mix well, transfer 700 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.

7. **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.

8. **Discard the filtrate, place the Spin Column back into the 2 ml collection tube, 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.

9. **Discard the filtrate, place the Spin Column back into the 2 ml collection tube, 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.

10. **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.

11. **Discard the 2 ml 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 13,000 rpm for 1 min.**

* If the centrifuge does not have a leak-proof cap, 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.

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