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Sarcocarp Total RNA Extraction Kit Instructions

Composition

Sarcocarp Total RNA Extraction Kit Cat. No.	5 Preps 5102005	50 Preps 5102050
Filter Columns	5	50
Spin Columns	5	50
β-mercaptoethanol	50 μl	500 μl
Buffer RLP	3 ml	30 ml
Buffer WA	1.9 ml	12 ml
Buffer WBR	1.5 ml	9.5 ml
RNase-free Water	1.5ml	2 ml×3
Instructions	1	1

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 than 2 years.

Technical Support

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

Introduction

This product does not involve the use of phenol chloroform and is suitable for the extraction of total RNA from 500 mg of highly saccharide and juicy plant tissues. The plant tissue lysate mixed with ethanol then added to the Spin Column, the RNA binds to the Spin Column, the RNA binds to the Spin Column, and the solubilized protein and PCR inhibitors are filtered out. After washing by two wash buffers, the RNA is eluted with RNase-free water, and 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. Liquid nitrogen, 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. Mortar, vortexer.
7. RNase-free labs.

Preparation Before Use

1. If the centrifuge has refrigeration function, set the temperature to 25℃.
2. Add 10 μl β-mercaptoethanol per 1 ml Buffer RLP and mix well. The addition of Buffer RLP with β-mercaptoethanol did not affect the experimental results after one month.
3. Add absolute ethanol to Buffer WA and Buffer WBR according to the instructions on the label the reagent bottle and tick the box on the label to mark "Ethanol Added".
4. Because saliva and skin contain RNases, latex gloves and masks are required during the whole process of RNA extraction.

Protocol

1. **Weigh 2~5 g sarcocarp tissue in a mortar, grind the tissue to powder form with liquid nitrogen, and then weigh 450~500 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.
* Do not use more than 500 mg of tissue, as this may cause clogging of the Filter Column and contamination of the purified RNA with genomic DNA.
* Buffer RLP is corrosive, please wear protective equipment when handling it.

2. **Add 500 µl Buffer RLP with β-mercaptoethanol, vortex and shake until the tissue is completely dissolved. Centrifuge at 13,000 rpm for 2 min.**
3. **Transfer 700 µl supernatant from step 2 to a Filter Column, close the lid, and centrifuge at 13,000 rpm for 1 min.**

* This step removes most of the genomic DNA and should not be omitted.
* If the lysate does not fully pass through the filter column, the nucleic acid content in the tissue is too high. At this time, transfer 300 µl filtrate to a clean 1.5 ml centrifuge tube, add 300 µl 70% ethanol to the 1.5 ml centrifuge tube and pipette 6~8 times to mix well, then transfer all the mixture to the Spin Column, close the lid, centrifuge at 13000 rpm for 1 min. Discard the filter column and residual filtrate, directly proceed to step 6.

4. **Discard the Filter Column, add 700 µl 70% ethanol to the filtrate and directly pipette 6~8 times to mix evenly, transfer 700 µl mixture to a Spin Column, close the lid, and centrifuge at 13000 rpm for 1 min.**

* If there is a precipitate in the mixture, please add the precipitate to the Spin Column.

5. **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.

6. **Discard the filtrate, place the Spin Column back into the 2 ml collection tube, add 500 µl Buffer WA, close the lid, and centrifuge at 13,000 rpm for 1 min.**

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

7. **Discard the filtrate, place the Spin Column back into the 2 ml collection tube, add 600 µl Buffer WBR, close the lid, and centrifuge at 13,000 rpm for 1 min.**

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

8. **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.

9. **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 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.

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

* Even if no DNA bands are visible by electrophoresis, extracted RNA are not free of genomic DNA contamination, and if complete DNA removal is required, digest the residual DNA with DNase I Column Digestion Kit (Simgen, Cat. No.8010050).