**Version No. FRL1903**

# FinePure Plant RNA Kit (Polysaccharides & Polyphenolics-rich)

## (Spin Column)

**Catalog No.: R302**

## Kit Content:

| Content | R302 (50 preps) |
|---|---|
| DNase I (2,000 U) | 1 bottle |
| DNase I Buffer | 4 ml |
| RNase Free ddH2O (tube) | 1 ml |
| 1ml RNase Free syringe | 1 pcs |
| Buffer RBL | 30 ml |
| Buffer RLP | 40 ml |
| Buffer RW | 40 ml |
| Buffer RW2 | 12 ml |
| RNase Free dd$H_2O$ (bottle) | 15 ml |
| RNAPure Spin Columns | 50 pcs |
| FinePure Filtration Columns | 50 pcs |
| 2 ml Collection Tubes | 2×50 pcs |
| 1.5 ml RNase Free Microcentrifuge Tubes | 50 pcs |

## Storage：

DNase I, DNase I Buffer and RNase-Free ddH2O in this kit should be transported at room temperature and stored at 2-8℃ on receipt. Others are stored at room temperature (15-25℃)。

**This Kit Special for Scientific Research.**

## Introduction

The unique lysis system adopted by the kit can extract the RNA from various simple plant tissue materials (leaves, stems, seedlings, etc.), especially polysaccharides & polyphenolics-rich plant tissue materials (fruits, seeds, etc.) and fungus and has wide scope of application. This kit can be used to effectively extract RNA with a molecular weight greater than 200 nt.

The kit is efficient, fast and convenient and does not require the extraction of phenol and chloroform in the extraction process. The RNA extracted with this kit is of high purity and is rarely contaminated by proteins, genomic DNA and other impurities. The kit can be used to purify high-purity RNA up to tens of micrograms from 50 ~ 100 mg plant tissues. The extracted RNA can be directly used in Northern hybridization, dot hybridization, mRNA purification, in vitro translation, protection analysis of RNA lytic enzyme, RT-PCR, RT-qPCR, cDNA library construction and other molecular biology experiments.

## To prevent RNase pollution, attention shall be paid to the following:

1. Change the gloves regularly, because the skin often carries bacteria, which can lead to RNase contamination.
2. Use RNase-free plastics and spear head to avoid cross contamination.
3. RNA is not degraded by RNase in Buffer RLP, but RNase-free plastics and glassware shall be used for further processing after extraction. The glassware can be baked at 150°C for 4 h and the plastic ware can be soaked in 0.5 M NaOH for 10 min, then thoroughly cleaned with water and sterilized to remove RNase.
4. RNase Free ddH2O shall be used to prepare solution. (Add the water to a clean glass bottle, add DEPC to final concentration 0.1%(v/v), mix well, place overnight and autoclave.)

## Precautions:

1. Before operation, add 50 μl β-mercaptoethanol to 1 ml Buffer RLP. The prepared RLT can be stored at 4°C for 1 month. Buffer RLP may form precipitates during storage. If precipitates appear, please heat and dissolve them at 60°C and use them after restoring to room temperature.
2. Whenever possible, use fresh collected samples. RNA yield is related to the integrity of the RNA in the initial sample, and RNA that has been degraded into small fragments according to the standard procedures in this manual cannot be effectively recovered.
3. Before the first use, add anhydrous ethanol to RW2 in the volume specified on the bottle label.
4. To extract the RNA containing Small RNA (<200 nt), ask GENFINE for the corresponding extraction process.

## Preparation of DNase I stock solution:

Absorb 550 µl RNase Free ddH2O with 1 ml RNase Free syringe first, inject it into a glass bottle containing DNase I dry powder (2,000 U), mix gently, dispense and store at -20°C (it can be stored for 9 months). (If required, purchase DNase I with Directory No.: FA102 separately) Note: DNase I stock solution melt at -20°C is stored at 4°C (can be stored for 6 weeks) and shall not be cryopreserved again.

## Operation Steps:

**Before use, add anhydrous ethanol to Buffer RW2 in the volume specified on the bottle.**

1. Column equilibration steps: add 500 µL Buffer RBL to the adsorption column RNAPure Spin Columns (put the adsorption column into the collection tube), centrifuge at 12,000 rpm for 2 min, dump the waste liquid in the collection tube, and put the adsorption column back into the collection tube. (Use the column treated on the same day)
2. Homogenization:

   Quickly transfer the fresh or cryopreserved plant tissue samples to a mortar precooled with liquid nitrogen, grind the tissues with a pestle and add liquid nitrogen continuously until the tissues are ground to powder **(no obvious visible particles. If the tissues are not ground completely, the yield and quality of RNA will be affected)**. Add the ground powder sample (50-100 mg) into a 1.5 mL sterile centrifuge tube containing 500 µL Buffer RLP **(check whether β-mercaptoethanol has been added before use)**, bring to vortex, shake vigorously and mix until there is no obvious precipitation in the lysate.

   **Note 1: For plant samples with an expected RNA yield of less than 10 µg, use an initial sample size of 100 mg. For starchy samples or mature leaves, increase Buffer RLP to 700 µL.**

   **Note 2: Due to rich plant diversity and different RNA content of the same plant in different growth and development stages and in different tissues, please choose the usage of appropriate plant materials according to the specific environment.**
3. Centrifuge at 12,000 rpm for 2 min.
4. Put FinePure Filtration Column in the collection tube, pipette the supernatant collected from centrifugation of last step to the filtration column, centrifuge at 12,000 rpm for 2 min and carefully pipette the filtrate in the collection tube to a new 1.5 ml RNase Free centrifuge tube. The sucker shall not contact with the cell debris precipitation in the collection tube.

5. Slowly add 0.4 times filtrate volume of anhydrous ethanol, mix well **(precipitation may appear at this point)**, transfer the solution and precipitation obtained to the adsorption column FineBind DNA Spin Columns **(the adsorption column is placed in the collection tube)**, centrifuge at 12,000 rpm for 15 sec, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
6. Add 350 µl Buffer RW to the adsorption column, centrifuge at 12,000 rpm for 1 min, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
7. Preparation of DNase I working solution: add 10 µl DNase I stock solution to a new RNase Free centrifuge tube, add 70 µl DNase I Buffer and mix gently.
8. Add 80 µl DNase I working solution to the center of the adsorption column and place at room temperature for 15 min.
9. Add 350 µl Buffer RW to the adsorption column, centrifuge at 12,000 rpm for 1 min, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube. 10. Add 500 µl Buffer RW2 **(check whether ethanol has been added before use)** to the adsorption column, place at room temperature for 2 min, centrifuge at 12,000 rpm for 1 min, discard the waste liquid in the collection tube and put the adsorption column back in the collection tube.
10. Repeat step 10 once.
11. Centrifuge at 12,000 rpm for 3 min, put the adsorption column in a new 1.5 ml RNase Free centrifuge tube, place at the room temperature for 2 min, add 50-100 µl RNase Free dd$H_2O$ to the center of the adsorption film, place at the room temperature for 1 min, and centrifuge at 12,000 rpm for 1 min to obtain RNA solution.

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