FavorPrep™
Plant Total RNA Mini Kit

User Manual

Cat. No.: FAPRK 001 (50 Preps)
FAPRK 001-1 (100 Preps)

For Research Use Only

Introduction

Plant Total RNA Mini Kit is specially designed for purification of total RNA from a variety of plant tissues. The method uses detergents and a chaotropic salt to lyse cell and inactivate RNase. In the presence of binding buffer with chaotropic salt, the total RNA in the lysate binds to glass fiber matrix in the spin column. The optional DNase treatments can remove DNA residues and the contaminants are washed with an ethanol contained wash buffer. Finally, the purified total RNA is eluted by RNase-free water. The protocol does not require phenol extraction and alcohol precipitation. The entire procedure can be completed in 60 minutes. The purified total RNA is ready for RT, RT-PCR, real-time PCR, Northern blotting. ssRNA and dsRNA of 200 bp to 1000's of bps in length are efficiently purified.

Quality Control

The quality of Plant Total RNA Mini Kit is tested on a lot-to-lot basis. The Kits are tested by isolation of total RNA from 25 mg young leaf. Purified RNA could be quantified with spectrophotometer and checked by agarose gel.

Sample Amount: up to 100 mg plant tissue or
1 X 10^7 plant cells
Format: spin column
Operation time: < 60 min
Yield: up to 100 µg
Elution volume: 50 µl

Kit Contents

	FAPRK001 (50preps)	FAPRK001-1 (100preps)
FARB Buffer	30 ml	60 ml
FAPRB Buffer	30 ml	60 ml
Wash Buffer 1	25 ml	60 ml
Wash Buffer 2 (conc.)*	15 ml	35 ml
RNase-free water	6 ml	6 ml
Filter column	50pcs	100pcs
FARB-Mini column	50pcs	100pcs
2ml collection tube	100pcs	200pcs

* For FAPRK001, add 60 ml ethanol(96-100%) to Wash Buffer 2.

* For FAPRK001-1, add 140 ml ethanol(96-100%) to Wash Buffer 2.

Caution

The component contains irritant agent. During operation, always wear a lab coat, disposable gloves, and protective goggles.

References

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Important notes

1. Make sure everything is RNase-free when handling RNA.

2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.

3. Pipet a required volume of FARB Buffer or FAPRB Buffer to another RNase-free container and add 10 µl β-mercaptoethanol (β-ME) per 1 ml FARB Buffer or FAPRB Buffer before use.

4. Add 60 ml (FAPRK001)/140 ml (FAPRK001-1) ethanol (96~100%) to Wash Buffer 2 when first open.

5. All centrifuge steps are done at full speed (14,000 rpm or 10,000 xg) in a microcentrifuge.

6. Dilute RNase-free DNase 1 in reaction buffer (1 M NaCl, 10 mM $MnCl_2$ 20 mM Tris-HCl, pH 7.0 at 25°C) to final conc. = 0.5 U/µl.

Plant RNA Mini Extraction Protocol

*Please Read Important Notes Before Starting The Following Steps.

1. Grind up to 100 mg plant sample under liquid nitrogen to a fine powder and transfer to a new microcentrifuge tube (not provided)

2. Add 450 µl of FARB Buffer (β-ME added) to the sample powder and vortex vigorously. Use FAPRB Buffer (β-ME added) if plant sample contains sticky secondary metabolites such as maize with milky endosperm or mycelia of filamentous fungi.

3. Place a Filter Column into a Collection Tube. And transfer the mixture to Filter Column then centrifuge for 2 min.

4. Transfer the clarified supernatant from the Collection Tube to a new microcentrifuge tube (not provided) and adjust the volume of the clear lysate.

5. Add 0.5 volume of ethanol (96 - 100%) to the clear lysate and mix by pietting.
   • For example, add 250 µl of ethanol to 500 µl of the clear lysate.

6. Place a FARB Mini Column into a Collection Tube. And transfer 750 µl of the ethanol added sample (including any precipitate) to FARB Mini Column. Centrifuge for 1 min and discard the flow-through.

7. Repeat step 6 for rest of the sample.

Plant RNA Mini Extraction Protocol

8. (Optional): To eliminate genomic DNA contamination, follow the steps from 8a. Otherwise, proceed to step 9 directly.
   8a. Add 250 µl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge for 1 min then discard the flow-through.
   8b. Add 100 µl of RNase-free DNase 1 solution (0.5 U/µl, not provided) to the membrane center of FARB Mini Column. Place the Column on the benchtop for 15 min.
   8c. Add 250 µl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge for 1 min then discard the flow-through.
   8d. After DNase 1 treatment, proceed to step 10.

9. Add 500 µl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge for 1 min then discard the flow-through.

10. Wash FARB Mini Column **twice** with 700 µl of Wash Buffer 2 by centrifuge for 1 min then discard the flow-through.
    • Make sure that ethanol has been added into Wash Buffer 2 when first open.

11. Centrifuge for an additional 3 min to dry the column.
    • Important Step! This step will avoid the residual liquid to inhibit the subsequent enzymatic reactions.

12. Place FARB Mini Column to a new microcentrifuge tube (not provided).

13. Add 50 µl of RNase-free ddH_2O to the membrane center of FARB Mini Column. Stand FARB Mini Column for 1 min.

14. Centrifuge for 2 min to elute RNA.

15. Store RNA at -70°C.

Protocol Technical Specifications

Different plant species contain a lot of different metabolites like polysaccharides, polyphenolics, lipids or proteins. Therefore, we provide two different lysis buffers for the various plant samples.

* The standard protocol uses FARB Buffer for lysis of plant sample. For most of common plant species, the buffer system ensures purified DNA with high yields and a good quality.

* Alternatively, buffer FAPRB is also provided with the kit. The different detergent in this lysis buffer is suitable for some plant sample with a lot of polysaccharides. In the majority of extractions both buffer systems should provide adequate results.