FavorPrep™
Plant Total RNA Mini Kit

User Manual

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

For Research Use Only
v.1101

Introduction

FavorPrep™ Plant Total RNA Extraction 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.

Sample amount and yield:

Sample Amount: up to 100 mg plant tissue or 1 X 10^7 plant cells
Operation time: About 30~60 min
Binding Capacity: up to 100 µg total RNA
Expected Yield: up to 5~30 µg total RNA from young leave
Elution volume: 50 µl

Kit Contents

Cat. No. / preps	FAPRK 001 (50 preps)	FAPRK 001-1 (100 preps)
FARB Buffer	30 ml	60 ml
FAPRB Buffer	30 ml	60 ml
Wash Buffer 1	30 ml	60 ml
Wash Buffer 2 (concentrated)	15 ml *	35 ml **
RNase-free Water	6 ml	6 ml
Filter Column	50 pcs	100 pcs
FARB Mini Column	50 pcs	100 pcs
Collection Tube	100 pcs	200 pcs
Elution Tube	50 pcs	100 pcs

* Add 60 ml ethanol (96-100 %) to Wash Buffer 2 when first open.
** Add 140 ml ethanol (96-100 %) to Wash Buffer 2 when first open.

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 hese buffers.

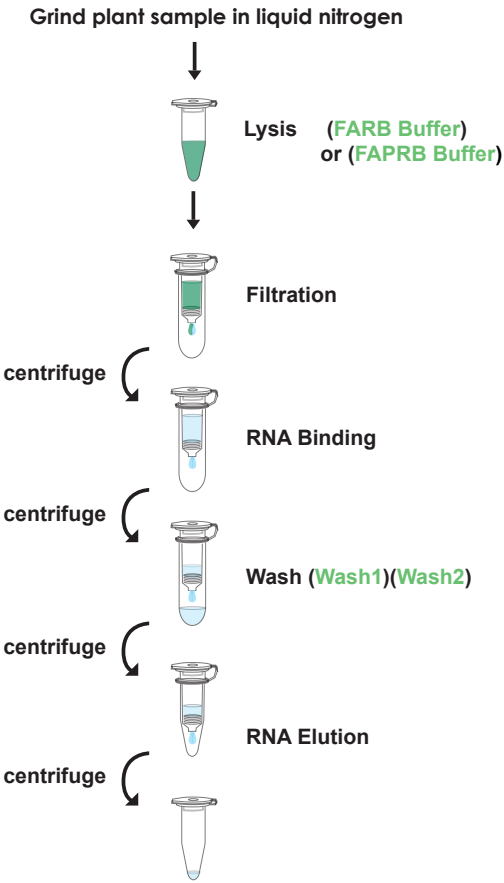
3. Pipet a required volume of FARB Buffer or FAPRB Buffer to another RNase- free container and add 10 µl ß-mercaptoethanol (ß-ME) per 1ml FARB Buffer or FAPRB Buffer before use.

4. Add required volume of RNase-free 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 x g) in a microcentrifuge.

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

Brief Procedure



Genernal 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).**
   --**Note**: Do not use plant sample more than 100 mg, it will lower the total RNA yield.

2. **Add 500 µl of FARB Buffer (ß-ME added) to the sample powder and vortex vigorously. Incubate at room temperature for 5 min. Use FAPRB Buffer (ß-ME added) if plant sample contains sticky secondary metabolites such as maize with milky endosperm or mycelia of filamentous fungi.**

   Note: In order to release all the RNA in the sample, it is required to disrupt the sample completely. Different samples require different methods (ex: disruptor equipment) to achieve complete disruption.

3. **Place a Filter Column to a Collection Tube and transfer the sample mixture to the Shearing Column. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 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.**
   --Avoid to pipette any debris and pellet from the Collection Tube.

5. **Add 1 volume of 70% ethanol to the clear lysate and mix well by vortexing.**

6. **Combine a FARB Mini Column with a Collection Tube. Transfer the ethanol added sample (including any precipitate) to the FARB Mini Column. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min, discard the flow-through and return the FARB Mini Column back to the Collection Tube.**

7. **Repeat step 6 for rest of the sample.**

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 the FARB Mini Column, centrifuge at full speed (14,000 rpm or 10,000 x g) for1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.

8b. Add 80 µl of RNase-free DNase 1 solution (0.5U/ul, not provided) to the membrane center of FARB Mini Column. Place the Column on the benchtop for 15 min.

8c. Add 250µl of Wash Buffer 1 to the FARB Mini Column, centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.

8d. After DNase 1 treatment, proceed to step 10.

9. Add 500 µl of Wash Buffer 1 to the FARB Mini Column, centrifugeat at full speed (14,000 rpm or 10,000 x g) for 1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.

10. Add 750 µl of Wash Buffer 2 to the FARB Mini Column, centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
--Make sure that ethanol has been added into Wash Buffer 2 when first open.

11. Repeat the step 15, add 750 µl of Wash Buffer 2 to the FARB Mini Column, centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.

12. Centrifuge the FARB Mini Column at full speed (14,000 rpm or 10,000 x g) for an additional 3 min to dry the FARB Mini Column.
--**Important Step!** This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.

13. Combine the FARB Mini Column with a Elution Tube.

14. Add 50 µl of RNase-free Water to the membrane center of the FARB Mini Column. Stand the FARB Mini Column for 1 min.
--**Important Step!** For effective elution, make sure that RNase-free Water is dispensed on the membrane center and is absorbed completely.

15. Centrifuge the FARB Mini Column at full speed (14,000 rpm or 10,000 x g) for 2 min to elute RNA.

16. Store RNA at -70C.