FavorPrep™
Plant Total RNA Maxi kit

User Manual

Cat. No.: FAPRK 002 (10 Preps)
FAPRK 002-1 (24 Preps)

For Research Use Only

v.1302

Introduction

FavorPrep™ Plant Total RNA Maxi Kit provides a fast and simple method to isolate total RNA from plant tissue and cells. In the process, sample is homogenized by grinding the plant tissue in liquid nitrogen and filtrated by filter column to remove cell debris. 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.

Sample amount and yield:

Sample Amount: 500 mg (up to 1 g) plant tissue or 5~10 X 10^7 plant cells

Operation time: about 45~60 min

Binding Capacity: up to 1000 µg total RNA

Expected Yield: up to 50~300 µg total RNA from young leave

Elution volume: 500 µl

Kit Contents

Cat. No. / preps	FAPRK 002 (10 preps)	FAPRK 002-1 (24 preps)
FARB Buffer	60 ml	140 ml
FAPRB Buffer	60 ml	140 ml
Wash Buffer 1	45 ml	140 ml
Wash Buffer 2 (concentrated)	12.5 ml *	50 ml **
RNase-free Water	6 ml	30 ml
Filter Column	10 pcs	24 pcs
FARB Maxi Column	10 pcs	24 pcs

* Add 50 ml ethanol (96-100 %) to Wash Buffer 2 when first open.
** Add 200 ml ethanol (96-100 %) to Wash Buffer 2 when first open.

Important notes

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

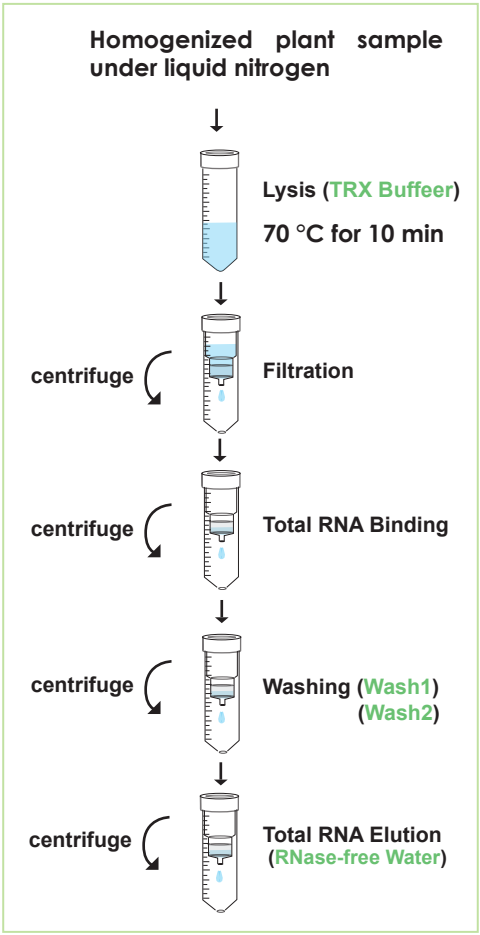
2. **Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.**

3. **Pipet 5 ml of FARB Buffer to another RNase-free container and add 50 µl of ß-mercaptoethanol (ß-ME) before every preparation.**

4. **Add required amount of ethanol (96-100%) Wash 2 Buffer when first open.**

5. **Dilute RNase-free DNase 1 in reaction buffer (1M NaCl, 10mM $MnCl_2$, 20mM Tris-HCl, pH7.0 at 25 °C) to final conc.= 0.5U/µl.**

Brief Procedure



Genernal Protocol:

Please Read Important Notes Before Starting The Following Steps.

1. **Grind 500 mg (up to 1 g) of plant sample under liquid nitrogen to a fine powder and transfer to a new 50 ml centrifuge tube (not provided).**
   --Note: Do not use plant sample more than 1g, it will lower the total RNA yield.

2. **Add 5 ml of FARB Buffer (ß-ME added) to the sample powder and vortex vigorously. 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. **Incubate at 70 °C for 10 min, vortex every 3 min during incubation.**

4. **Place a Filter Column to a 50 ml centrifuge tube (not provided). And transfer the entire sample mixture to the Filter Column.**

5. **Centrifuge at full speed (4500~6,000 rpm) for 5 min at 4 °C.**

6. **Transfer the clarified flow-through to a new 50 ml centrifuge tube (not provided) and adjust the volume of the clarified flow-through.**
   --Avoid pipett any debris and pellet when transfering the clarified flow-through.

7. **Add 1 volume of 70 % ethanol to the clarified flow-through and mix well by plus-vortexing for 5 seconds.**
   --For example, add 4.5 ml of 70 % ethanol to 4.5 ml of clearified flow-through.

8. Place a FARB Maxi Column to a 50 ml centrifuge tube (not provided). Transfer the ethanol added sample mixture (including any precipitate) to the FARB Maxi Column. Centrifuge at full speed (4500~6,000 rpm) for 1 min. Discard the flow-through and place the FARB Maxi Column back to the 50 ml centrifuge tube.

9.(Optional): To eliminate genomic DNA contamination of RNA, follow the steps from 9a. Otherwise, proceed to step 10 directly.

9a. Add 2.5 ml of Wash 1 Buffer to the FARB Maxi Column. Centrifuge at full speed (4500~6,000 rpm) for 2 min. Discard the flow-through and place the FARB Maxi Column back to the 50 ml centrifuge tube.

9b. Add 800 µl of RNase-free DNase 1 solution (0.5 U/µl, not provided) to the membrane center of FARB Maxi Column. Place the Column on the benchtop for 15 min.

9c. Add 2.5 ml of Wash 1 Buffer to the FARB Maxi Column. Centrifuge at full speed (4500~6,000 rpm) for 2 min. Discard the flow-through and place the FARB Maxi Column back to the 50 ml centrifuge tube.

9d. After DNase 1 treatment, proceed to step 11.

10. Add 5 ml of Wash 1 Buffer to wash the FARB Maxi Column, Centrifuge at full speed (4500~6,000 rpm) for 2 min. Discard the flow-through and place the FARB Maxi Column back to the 50 ml centrifuge tube.

11. Wash FARB Maxi Column *twice* with 5 ml of Wash 2 Buffer by Centrifuge at full speed (4500~6,000 rpm) for 2 min. Discard the flow-through and place the FARB Maxi Column back to the 50 ml centrifuge tube.
--Make sure that ethanol has been added into Wash 2 Buffer when first open.

12. Centrifuge at full speed (4500~6,000 rpm) for an additional 10 min to dry the FARB Maxi column.
--Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.

13. Place the FARB Maxi Column to a new 50 ml centrifuge (not provided).

14. Add 1 ml of RNase-free Water to the membrane center of the FARB Maxi Column. Stand the FARB Maxi Column for 5 min.
--Important Step! For effective elution, make sure that the elution solution is dispensed of the membrane center and is absorbed completely.

15. Centrifuge at full speed (4500~6,000 rpm) for 5 min to elute RNA.

16. Store RNA at -70 °C.