FavorPrep™ Plant Total RNA Maxi Kit

Cat. No.: FAPRK 002

(For Research Use Only)

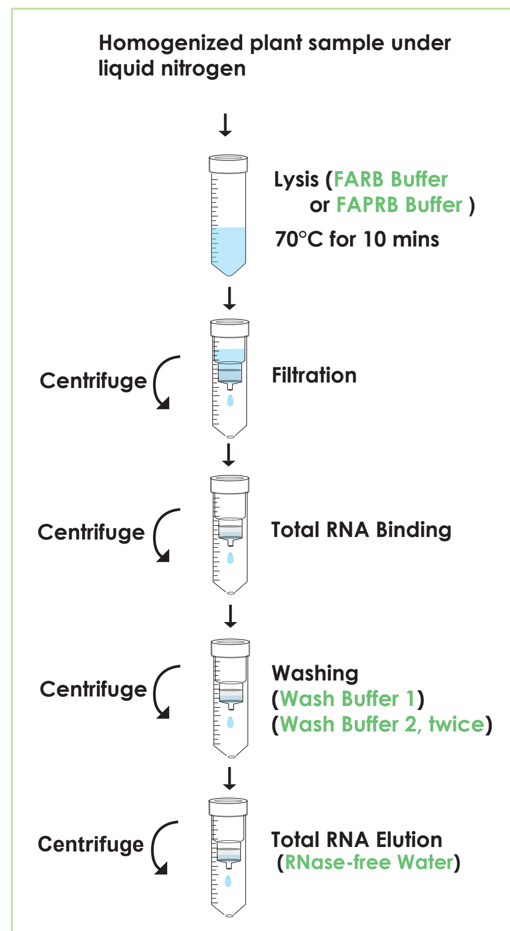
Introduction

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 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 mins. The purified total RNA is ready for RT, RT-PCR, real-time PCR, Northern blotting.

Kit Contents:

Cat. No:	FAPRK 002 (10 preps)
FARB Buffer	60 ml
FAPRB Buffer	60 ml
Wash Buffer 1	60 ml
Wash Buffer 2 (Concentrate)[a]	12.5 ml
RNase-free Water	6 ml
Filter Columns	10 pcs
FARB Maxi Columns	10 pcs
User Manual	1
Preparation of Wash Buffer 2 by adding ethanol (96~100%)	
Ethanol volume for Wash Buffer 2 [a]	50 ml

Brief procedure:



Specification:

Principle: maxi spin column (silica matrix)
Sample size: up to 1 g plant tissue or 5~10×10^7 plant cells
Operation time: 45~60 mins
Binding capacity: up to 2000 µg total RNA/column
Expected yield: 50~300 µg of total RNA from 1 g of young leave
Column applicability: centrifugation and vacuum
Minimum elution volume: 500 µl

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 50 µl ß-mercaptoethanol (ß-ME) per 50 ml FARB Buffer or FAPRB Buffer before use. **Caution: ß-mercaptoethanol is hazardous to human health. perform the procedures involving FARB Buffer or FAPRB Buffer in a chemical fume hood.**
4. Add required volume of RNase-free ethanol (96~100%) to Wash Buffer 2 at the first use.
5. Use a centrifuge with a swinging bucket rotor for 50 ml tube in all centrifugation steps. The maximum speed should be 4,500~6,000 rpm.
6. Dilute RNase-free DNase I 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.

General Protocol:

Please Read Important Notes Before Starting 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 1 g, it will be 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 mins, vortex every 3 mins 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 (4,500~6,000 rpm) for 5 mins 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 pipette any debris and pellet when transferring the clarified flow-through.
7. Add 1X volume of 70% ethanol to the clarified flow-through and mix well by plus-vortexing for 5 secs.
   -For example, add 4.5 ml of 70% ethanol to 4.5 ml of clarified 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 (4,500~6,000 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 Buffer 1 to the FARB Maxi Column. Centrifuge at full speed (4500~6,000 rpm) for 2 mins. 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 I solution (0.5 U/µl, not provided) to the membrane center of FARB Maxi Column. Place the Column on the benchtop for 15 mins.
9c. Add 2.5 ml of Wash Buffer 1 to the FARB Maxi Column. Centrifuge at full speed (4500~6,000 rpm) for 2 mins. Discard the flow-through and place the FARB Maxi Column back to the 50 ml centrifuge tube.
9d. After DNase I treatment, proceed to step 11.

10. Add 5 ml of Wash Buffer 1 to wash the FARB Maxi Column, Centrifuge at full speed (4,500~6,000 rpm) for 2 mins. 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 Buffer 2 by Centrifuge at full speed (4,500~6,000 rpm) for 2 mins. 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 Buffer 2 at the first open.

12. Centrifuge at full speed (4,500~6,000 rpm) for an additional 10 mins 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 mins.
    -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 (4,500~6,000 rpm) for 5 mins to elute RNA.

16. Store RNA at -70°C.