FavorPrep™ Plant Total RNA Purification Maxi Sample Kit

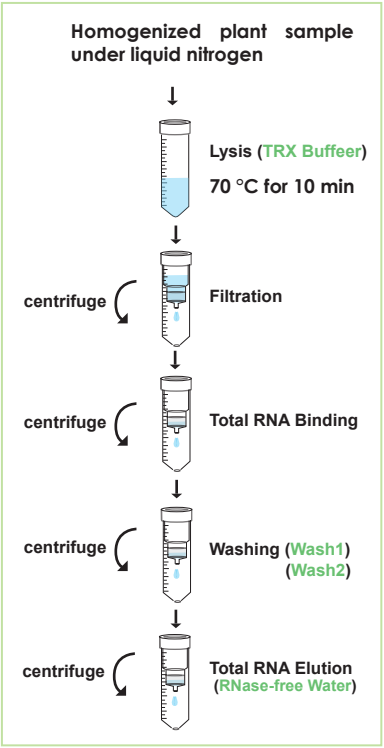
(Cat.: FAPRK 000-Maxi, 2 Preps)
(For Research Use Only) v.1012

Kit Contents

	FAPRK 000-Maxi (2 Preps)
FARB Buffer	12 ml
FAPRB Buffer	12 ml
Wash Buffer 1	10 ml
Wash Buffer 2* (concentrated)	5 ml
RNase-free water	1 ml
Filter Column	2 pcs
FARB Maxi Column	2 pcs

* Add 20 ml of RNase-free ethanol (96-100 %) to Wash Buffer 2 when first open.

Brief Procedure



Specifications

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

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 Buffer 2 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.

General 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 Buffer 1 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 Buffer 1 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 Buffer to 1 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 Buffer 2 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.