FavorPrep™ Plant Total RNA Mini Kit (for woody plant)

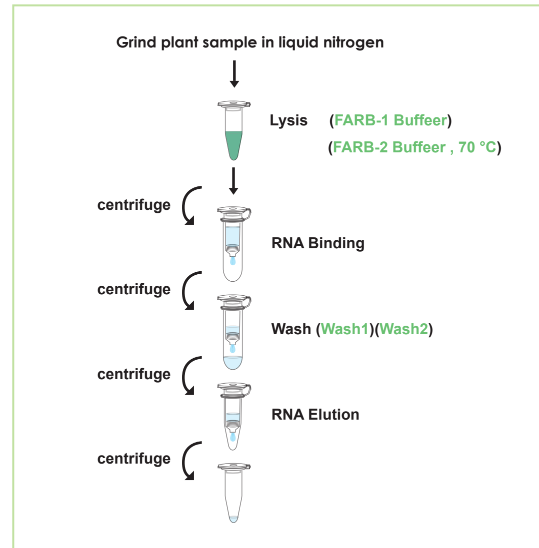
Cat.: FAPRK 003 (50 Preps)
FAPRK 003-1 (100 Preps)
(For Research Use Only) v.1005

Kit Contents

	FAPRK 003 (50 preps)	FAPRK 003-1 (100 preps)
FARB-1 Buffer	30 ml	60 ml
FARB-2 Buffer	4 ml	8 ml
Wash Buffer 1	30 ml	60 ml
Wash Buffer 2 (conc.)*	15 ml	35 ml
RNase-free water	6 ml	6 ml
FARB Mini Column	50 Pcs	100 Pcs
2ml Collection Tube	50 Pcs	100 Pcs

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

Brief Procedure



Specifications

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

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-1 Buffer to another RNase-free container and add 10 µl of ß-mercaptoethanol (ß-ME) per 1ml FARB-1 Buffer before use.
4. Add required volume of ethanol(96-100%) as bottle indicated to 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 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 500 µl of FARB-1 Buffer (ß-ME added) to the sample powder and vortex vigorously.

> 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.Add 50 µl of FARB-2 Buffer and incubate at 70 °C for 10 min, vortex every 3 min during incubation.

4. Centrifuge at 12,000 rpm for 5 min at 5 °C.

5.Transfer the clarified supernatant to a new microcentrifuge tube (not provided) and adjust the volume of the clear lysate.
--Avoid pipett any debris and pellet from the Collection Tube.

6.Add 0.9 volume of ethanol (96-100%) to the clear lysate and mix well.
--For example, add 450 µl of ethanol to 500 µl of clear lysate.

7.Place a FARB Mini Column into a Collection Tube, and transfer 750 µl of the ethanol added sample mixture (including any precipitate) to FARB Mini Column. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min and discard the flow-through.

8.Repeat step 7 for rest of the sample mixture.

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

9a. Add 250 µl of Wash Buffer 1 to FARB Mini Column, Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.
9b. Add 60 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.
9c. Add 250 µl of Wash Buffer 1 to FARB Mini Column. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discaard the flow-through.
9d. After DNase 1 treatment, proceed to step 11.

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

11.Wash FARB Mini Column twice with 750 µl of Wash Buffer 2 by centrifuge at full speed (14,000 rpm or 10,000 x g) for1 min then discard the flow-through.
--Make sure that ethanol has been added into Wash Buffer 2 when first open.

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

13.Place FARB Mini Column to Elution Tube.

14. Add 50 µl of RNase-free ddH2O to the membrane center of FARB Mini Column. Stand FARB Mini Column for 1 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 (14,000 rpm or 10,000 x g) for 2 min to elute RNA.

16. Store RNA at -70 °C.

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