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

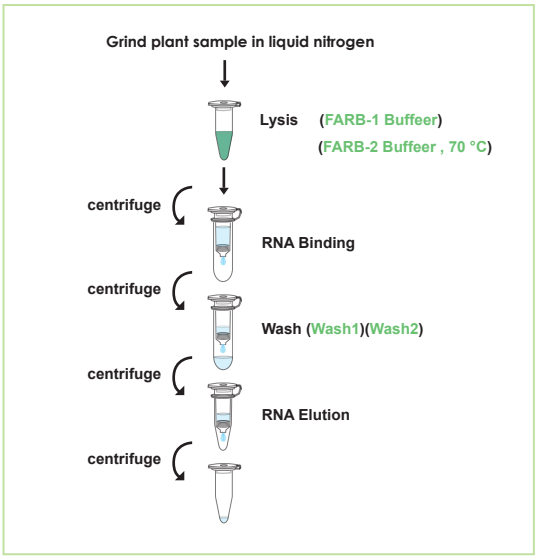
Cat.: FAPRK 003 (50Preps)
FAPRK 003-1 (100Preps)
(For Research Use Only)

## Kit Contents

| | FAPRK 003 | FAPRK 003-1 |
|---|---|---|
| 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 |
| 2 ml Collection Tube | 50 Pcs | 100 Pcs |

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

## Brief Procedure




## 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 ul of B-mercaptoethanol (B-ME) per 1ml FARB-1 Buffer before use.
4. Add required amount 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 MnCl2, 20mM Tris-HCl, pH7.0 at 25 °C) to final conc.= 0.5U/ul.

## 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 ul of FARB-1 Buffer (B-ME added) to the sample powder and vortex vigorously.

3.Add 50 ul 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 ul of ethanol to 500 ul of clear lysate.

**7.Place a FARB Mini Column into a Collection Tube, And transfer 750 ul 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 ul 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 100 ul of RNase-free DNase 1 solution (0.5 U/ul, not provided) to the membrane center of FARB Mini Column. Place the Column on the benchtop for 15 min.**
**9c. Add 250 ul 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 ul 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 ul 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 ul of RNase-free dd$H_2O$ 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.**