

Kit Contents:
(For Research Use Only)

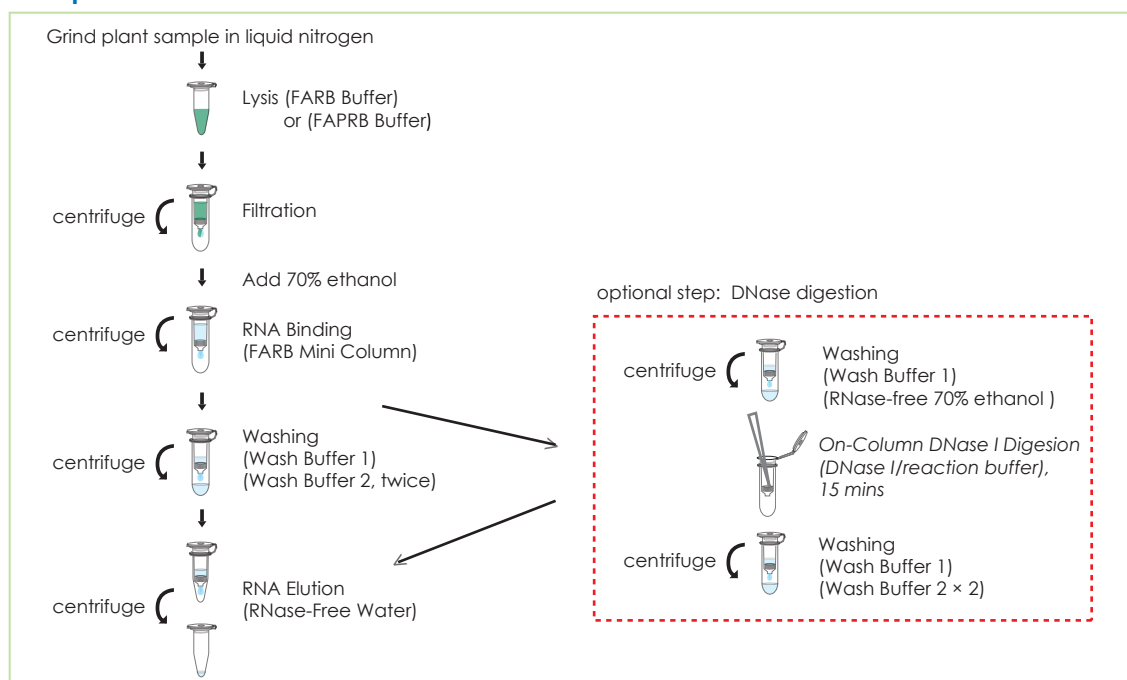
Cat. No:	FAPR1020 (4 preps)	FAPR1023 (50 preps)	FAPR1024 (100 preps)	FAPR1026 (300 preps)
FARB Buffer	1.5 ml × 2	30 ml	60 ml	170 ml
FAPRB Buffer	1.5 ml × 2	30 ml	60 ml	170 ml
Wash Buffer 1	1.5 ml × 2	30 ml	60 ml	170 ml
Wash Buffer 2 (Concentrate) ^a	1.5 ml	20 ml	35 ml	50 ml × 2
RNase-Free Water	0.5 ml	6 ml	6 ml	8 ml × 2
Filter Columns	4 pcs	50 pcs	100 pcs	300 pcs
FARB Mini Columns	4 pcs	50 pcs	100 pcs	300 pcs
Collection Tubes	8 pcs	100 pcs	200 pcs	600 pcs
Elution Tubes	4 pcs	50 pcs	100 pcs	300 pcs
User Manual	1	1	1	1
Preparation of Wash Buffer 2 by adding ethanol (96~100%)				
Ethanol volume for Wash Buffer 2 ^a	6 ml	80 ml	140 ml	200 ml

Specification:

Principle: mini spin column (silica matrix)
 Sample size: up to 100 mg plant tissue or 1×10^7 plant cells
 Operation time: <60 mins
 Binding capacity: up to 100 µg total RNA/column
 Expected yield: 5~30 µg of total RNA from 100 mg of young leave
 Column applicability: centrifugation and vacuum
 Minimum elution volume: 30 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 10 µl β-mercaptoethanol (β-ME) per 1 ml FARB Buffer or FAPRB Buffer before use. **Caution: β-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 when first use.
5. All centrifuge steps are done at full speed (~18,000 xg) in a microcentrifuge.
6. (Optional) For each reaction, prepare 60 µl of RNase-free DNase I solution (0.25 U/µl). Prepare a 10× DNase I reaction buffer containing 1 M NaCl, 10 mM MnCl₂ or MgCl₂, and 20 mM Tris-HCl (pH 7.0 at 25°C). Dilute this buffer to a 1× working concentration before use. Use the 1× buffer to dilute the DNase I enzyme to a final concentration of 0.25 U/µl. Alternatively, use the ready-to-use **FavorPrep™ DNase I Solution (Cat. No. FAD12093)** to simplify preparation.

Brief procedure:


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).
-Note: Do not use plant sample more than 100 mg, it will lower the total RNA yield.
2. Add 500 µl of FARB Buffer (β-ME added) to the sample powder and vortex vigorously. Incubate at room temperature for 5 mins. Use FAPRB Buffer (β-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 from sample, it is required to disrupt the sample completely by using a suitable disruptor equipment.
3. Place a Filter Column to a Collection Tube and transfer the sample mixture to the Filter Column. Centrifuge at full speed (~18,000 xg) for 1 min.
4. Transfer the clarified supernatant from the Collection Tube to a new microcentrifuge tube (not provided), and adjust the volume of the supernatant.
-Note: Avoid to pipette any debris and pellet when transferring the supernatant.
5. Add 1 volume of RNase-free 70% ethanol and mix well by vortexing.
6. Place a FARB Mini Column to a Collection Tube and transfer the ethanol added sample mixture (including any precipitate) to the FARB Mini Column. Centrifuge at full speed for 1 min, discard the flow-through and return the FARB Mini Column back to the Collection Tube.
7. Repeat step 6 for the rest of the sample mixture.
8. (Optional): To eliminate genomic DNA contamination, follow the steps from 8a. Otherwise, proceed to step 9 directly.

- 8a. Add 250 µl of Wash Buffer 1 to the FARB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
 - 8b. Add 750 µl of RNase-free 70% ethanol to the FARB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
 - 8c. Add 60 µl of RNase-free DNase I solution (0.25 U/µl, not provided) to the membrane center of FARB Mini Column. Place the column on the benchtop for 15 mins.
 - 8d. Add 250 µl of Wash Buffer 1 to the FARB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
 - 8e. After DNase I treatment, proceed to step 10.
9. Add 500 µl of Wash Buffer 1 to the FARB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
10. Add 750 µl of Wash Buffer 2 to the FARB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
-Note: Make sure that ethanol has been added into Wash Buffer 2 when first use.
11. Repeat step 10 for one more washing.
12. Centrifuge the FARB Mini Column at full speed for an additional 3 mins to dry the FARB Mini Column.
-Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.
13. Place the FARB Mini Column to a Elution Tube (provided, 1.5 ml microcentrifuge tube).
14. Add 30~50 µl of RNase-Free Water to the membrane center of the FARB Mini Column. Stand the FARB Mini Column for 1 min.
-Important Step! For effective elution, make sure that RNase-Free Water is dispensed on the membrane center and is absorbed completely.
-Important : Do not elute the RNA using RNase-free water less than suggested volume (<30 µl). It will lower the final yield.
15. Centrifuge the FARB Mini Column at full speed for 1 min to elute RNA.
16. Store RNA at -70°C.