FavorPrep™ Plant Total RNA Mini Kit

Cat. No.: FAPRK 000
FAPRK 001
FAPRK 001-1
FAPRK 001-2
(For Research Use Only)

Kit Contents:

Cat. No:	FAPRK 000-Mini (4 preps_sample)	FAPRK 001 (50 preps)	FAPRK 001-1 (100 preps)	FAPRK 001-2 (300 preps)
FARB Buffer	3 ml	30 ml	60 ml	170 ml
FAPRB Buffer	3 ml	30 ml	60 ml	170 ml
Wash Buffer 1	3 ml	30 ml	60 ml	170 ml
Wash Buffer 2 (concentrate)[a]	1.5 ml	20 ml	35 ml	50 ml x 2
RNase-free Water	0.5 ml	6 ml	6 ml	8 ml x 2
Filter Column	4 pcs	50 pcs	100 pcs	300 pcs
FARB Mini Column	4 pcs	50 pcs	100 pcs	300 pcs
Collection Tube	8 pcs	100 pcs	200 pcs	600 pcs
Elution Tube	4 pcs	50 pcs	100 pcs	300 pcs
User Manual	1	1	1	1
Preparation of Wash Buffer by adding ethanol (96 ~ 100%)				
Ethanol volume for Wash Buffer[a]	6 ml	80 ml	140 ml	200 ml

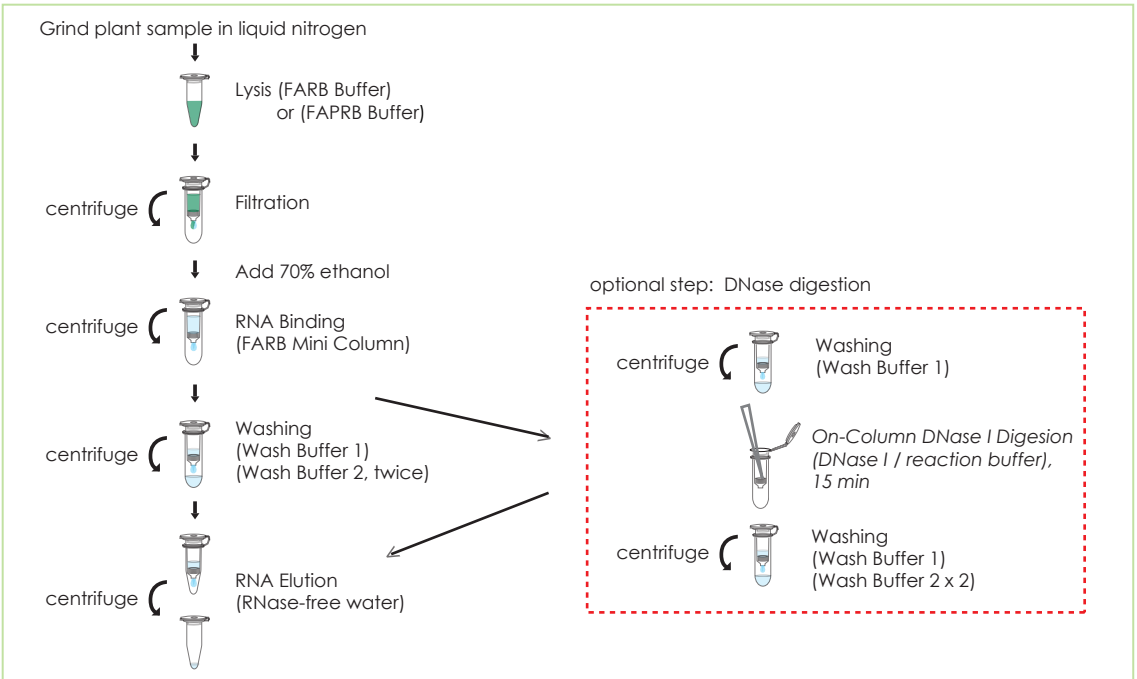
Specification:

Principle: mini spin column (silica matrix)
Sample size: up to 100 mg plant tissue or 1 x10^7 plant cells
Operation time: 30 ~ 60 minutes
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 ß-mercaptoethanol (ß-ME) per 1ml 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 when first use.
5. All centrifuge steps are done at full speed (~18,000 x g) in a microcentrifuge.
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.

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 (ß-ME added) to the sample powder and vortex vigorously. Incubate at room temperature for 5 min. 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 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 x g) 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 70 % RNase-free 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 for1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
   8b. Add 60 µl of RNase-free DNase I solution (0.5 U/µl, not provided) to the membrane center of FARB Mini Column. Place the column on the benchtop for 15 min.
   8c. 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.
   8d. After DNase I treatment, proceed to step 10.

9. Add 500 µl of Wash Buffer 1 to the FARB Mini Column, centrifugeat 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 min 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 ddH_2O 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 ddH_2O 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.