FavorPrep™ Plant Total RNA Extraction HE Mini Kit

Kit Contents

Cat. No.	FAPR1030 (4 Preps)	FAPR1033 (50 Preps)	FAPR1034 (100 Preps)
PR1 Buffer	5 ml	50 ml	100 ml
PR2 Buffer	3 ml	30 ml	60 ml
Wash Buffer 1	5 ml	60 ml	110 ml
Wash Buffer 2 (Concentrate)▲	1.5 ml	20 ml	35 ml
RNase-Free Water	0.5 ml	6 ml	8 ml
Micropestles	4 pcs	50 pcs	50 pcs x 2
Filter Columns	4 pcs	50 pcs	50 pcs x 2
HE Columns	4 pcs	50 pcs	50 pcs x 2
HE Collection Tubes	8 pcs x 2	100 pcs x 2	100 pcs x 4
Elution Tubes	4 pcs	50 pcs	100 pcs
User Manual	1	1	1
Preparation of **Wash Buffer 2** by adding 96~100% ethanol.			
Volume of Ethanol for Wash Buffer 2▲	6 ml	80 ml	140 ml

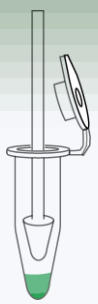
All kit components are shipped at room temperature and should be stored at room temperature between 15~25°C upon receipt.

Specification

Format/Principle	Spin Column (silica matrix)
Binding Capacity	≤200 μg RNA/Column
Operation Time	<45 mins
Sample Size	≤125 mg (fresh weight)/≤25 mg (dry weight)
RNA yield	≤180 μg
Elution Volume	30 μl

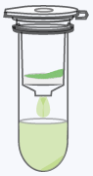
Procedure Overview

Plant sample



- Weigh plant sample and grind it into fine powder.
- Add 850 µl PR1-β-ME mixture and vortex.
- Incubate at 65°C for 10 mins.

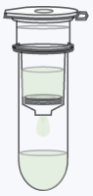
Filter Column



Centrifuge
18,000 xg, 3 mins

- Transfer entire mixture into Filter Column.

HE Column



Centrifuge
18,000 xg, 1 min

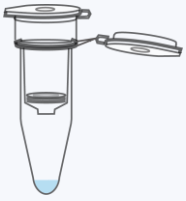
- Transfer all the supernatant of filtrate into an HE Collection Tube.
- Add 500 µl PR2 Buffer and 700 µl ethanol (96~100%), mix well.
- Transfer 1000 µl of the mixture into HE Column and centrifuge.
- Repeat for the remaining mixture.

Centrifuge
18,000 xg, 1 min

- Add 900 µl Wash Buffer 1 and centrifuge.
- Add 900 µl Wash Buffer 2 (ethanol contained) and centrifuge.

Centrifuge
18,000 xg, 2 mins

- Add 500 µl Wash Buffer 2 (ethanol contained) and dry the column membrane.



Centrifuge
18,000 xg, 2 mins

- Add 30 µl RNase-Free Water.
- Stand the column for 5 mins.
- Obtain purified RNA.

■ Preparation Before Starting

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. Additional materials: RNase-free 70%, 96~100% ethanol and β-Mercaptoethanol (β-ME).
4. (Optional) For long-term RNA storage, immerse the plant tissue in FavorPrep™ NApreserve Reagent (Cat. No. FNPR1084) as instructed in the user manual.
5. (Optional) Prepare DNase I working solution following the user guide of FavorPrep™ DNase I Solution (Cat. No. FADI209) and make the final concentration of DNase I to 0.25 U/μl.
6. Set up a water bath or dry bath at 65°C.
7. Check **PR1 Buffer** before use. If precipitates are observed, vortex PR1 Buffer until precipitates are completely dissolved.
8. For a fresh preparation of **PR1-β-ME mixture**, premix 850 μl of **PR1 Buffer** and 20 μl of β-ME per sample before executing RNA extraction.
9. **Caution: β-ME is hazardous to human health. Always perform procedures involving β-ME in a fume hood.**
10. Add indicated volume of ethanol (96~100%) into **Wash Buffer 2**, mix well and store at room temperature.

■ General Protocol

- **Note:** All centrifugation steps should be performed at **18,000 xg** at room temperature.
- **Note:** Avoid disturbing the pellet or debris while transferring the supernatant.

1. Grind 50 mg of wet weight (up to 125 mg) plant tissue or 20 mg (up to 50 mg) dry weight of plant tissue under liquid nitrogen to a fine powder and transfer to a new microcentrifuge tube (not provided).
   - Do not allow the sample to thaw and continue immediately to step 2.
2. Add 850 μl **PR1-β-ME mixture** to the tube and vortex thoroughly.
3. Incubate mixture at 65°C for 10 mins and vortex occasionally during incubation.
   - If the mixture gradually turns brown, stop the incubation process and proceed to step 4.
4. Place a **Filter Column** to an **HE Collection Tube** and transfer the entire mixture to the Filter Column.
5. Centrifuge for 3 mins, then transfer all the supernatant of filtrate carefully into a new HE Collection Tube.

6. Add 500 µl **PR2 Buffer** and mix thoroughly by pipetting.
7. Add 700 µl ethanol (96~100%) and mix thoroughly by pipetting.
8. Place an **HE Column** in a new HE Collection Tube.
9. Transfer 1000 µl mixture carefully into the HE Column and centrifuge for 1 min. Discard flow-through.
10. Repeat step 9 for the remaining mixture and place the HE Column in a new HE Collection Tube.
11. **(Optional) DNase I digestion.** To eliminate genomic DNA contamination, follow the steps from a.
    a. Add 450 µl of **Wash Buffer 1** to the HE Column, and centrifuge at 18,000 xg for 1 min. Discard the flow-through and place the HE Column in the HE Collection Tube.
    b. Add 900 µl of **RNase-free 70% ethanol** to the HE Column, and centrifuge at 18,000 xg for 1 min. Discard the flow-through and place the HE Column in the HE Collection Tube.
    c. Add 60 µl of **RNase-free DNase I solution** (0.25 U/µl, not provided) to the membrane center of the HE Column. Place the column on the benchtop for 15 mins.
    d. Add 450 µl of **Wash Buffer 1** to the HE Column , and centrifuge at 18,000 xg for 1 min. Discard the flow-through and place the HE Column in the HE Collection Tube.
    e. Proceed to step 13.
12. Add 900 µl **Wash Buffer 1** to the HE Column. Centrifuge for 1 min then discard flow-through.
13. Add 900 µl **Wash Buffer 2** (ethanol contained) to the HE Column. Centrifuge for 1 min then discard flow-through.
14. Add 500 Wash Buffer 2 (ethanol contained) to the HE Column. Centrifuge for 2 mins to dry the membrane directly. Discard flow-through and HE Collection Tube.
15. Place the HE Column in an **Elution Tube**, then add 30 µl **RNase-Free Water** directly onto the membrane. Stand the HE Column for 5 mins.
    - **Important step!** For effective elution, ensure that the elution solution is dispensed onto the membrane center and absorbed completely.
16. Centrifuge for 2 mins to elute RNA.