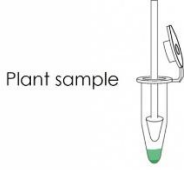

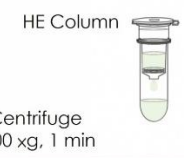





The Plant Total RNA Extraction HE Mini Kit is an efficient tool to rapidly isolate high-purity, high-concentration total RNA from a wide variety of plant types, including bryophytes, pteridophytes, gymnosperms, and angiosperms. It also enables reliable RNA extraction from various plant organs without interference from polyphenols or polysaccharides.

The distinctive HE Column allows to enlarge sample input and reduce elution volume to obtain both higher eluted RNA concentration and yield. Optimized washing steps effectively eliminate contaminants, inhibitors, and salts, resulting in an excellent OD 260/230 ratio. The purified RNA is suitable for a variety of downstream applications, including studies on genetic diversity, phylogenetics, molecular breeding, synthetic biology, and small RNA-mediated gene regulation, among others.

<p><b>Specifications:</b></p> <p>Format/Principle: Spin Column (silica matrix)</p> <p>Binding Capacity: ≤200 µg RNA/Column</p> <p>Operation Time: &lt;45mins</p> <p>Sample Size: ≤125 mg(fresh weight) ≤ 25 mg(dry weight)</p> <p>RNA yield: ≤180 µg</p> <p>Elution Volume: 30 µl</p>	 <p>Plant sample</p>	<ul style="list-style-type: none"> <li>• Weigh plant sample and grind it into fine powder.</li> <li>• Add 850 µl PR1-β-ME mixture and vortex.</li> <li>• Incubate at 65°C for 10 mins.</li> </ul>
	 <p>Filter Column</p> <p>Centrifuge 18,000 xg, 3 mins</p>	<ul style="list-style-type: none"> <li>• Transfer entire mixture into Filter Column.</li> </ul>
	 <p>HE Column</p> <p>Centrifuge 18,000 xg, 1 min</p>	<ul style="list-style-type: none"> <li>• Transfer all the supernatant of filtrate into an HE Collection Tube.</li> <li>• Add 500 µl PR2 Buffer and 700 µl ethanol (96~100%), mix well.</li> <li>• Transfer 1000 µl of the mixture into HE Column and centrifuge.</li> <li>• Repeat for the remaining mixture.</li> </ul>
	 <p>Centrifuge 18,000 xg, 1 min</p>	<ul style="list-style-type: none"> <li>• Add 900 µl Wash Buffer 1 and centrifuge.</li> <li>• Add 900 µl Wash Buffer 2 (ethanol contained) and centrifuge.</li> </ul>
	 <p>Centrifuge 18,000 xg, 2 mins</p>	<ul style="list-style-type: none"> <li>• Add 500 µl Wash Buffer 2 (ethanol contained) and dry the column membrane.</li> </ul>
	 <p>Centrifuge 18,000 xg, 2 mins</p>	<ul style="list-style-type: none"> <li>• Add 30 µl RNase-Free Water.</li> <li>• Stand the column for 5 mins.</li> <li>• Obtain purified RNA.</li> </ul>

Kit components	RE-2103-HE	RE-2103-50HE	RE-2103-100HE
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(Conc.) ▲	1.5 ml	20 ml	35 ml
RNase free Water	0,5 ml	6 ml	8 ml
Micropestles	4 pcs	50 pcs	50 pcs × 2
Filter Columns	4 pcs	50 pcs	50 pcs x 2
HE Columns	4 pcs	50 pcs	50 pcs × 2
HE Collection Tubes	8 pcs × 2	100 pcs × 2	100 pcs × 4
Elution Tubes	4 pcs	50 pcs	100 pcs
Preparation of Wash Buffer by adding 96~100% ethanol.			
Volume of Ethanol for Wash Buffer ▲	6 ml	80 ml	140 ml

- **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  $\beta$ -Mercaptoethanol ( $\beta$ -ME).
4. (Optional) For long-term RNA storage, immerse the plant tissue in our preservation Reagent to analyze later.
5. (Optional) Prepare DNase I working solution following the user guide of DNase I solution and make the final concentration of DNase I to 0.25 U/ $\mu$ l.
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- $\beta$ -ME mixture, premix 850  $\mu$ l of PR1 Buffer and 20  $\mu$ l of  $\beta$ -ME per sample before executing RNA extraction.
9. Caution:  $\beta$ -ME is hazardous to human health. Always perform procedures involving  $\beta$ -ME in a fume hood.
10. Add indicated volume of ethanol (96~100%) into Wash Buffer 2, mix well and store at room temperature.

**Recommendations for a later analysis**

If the samples cannot be processed immediately after collection, we suggest performing appropriate pre-treatment steps prior to storage to prevent RNA degradation:

1. Rinse the surface of the marine samples with RNase-free water.
2. Quickly freeze the samples in liquid nitrogen and store them at -80 °C.
3. If liquid nitrogen is not available, cut the samples into small pieces and soak them in Preservation Reagent , then store at -20 °C.

To obtain high-quality and intact RNA from marine plants, thorough disruption during the lysis step is essential.

We recommend grinding the samples in liquid nitrogen and ensuring they are processed into a fine powder while avoiding thawing.

**■ General Protocol**

-Note: All centrifugation steps should be performed at 18,000 ×g 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 of 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 of PR2 Buffer and mix thoroughly by pipetting.

7. Add 700 µl of ethanol (96~100%) and mix thoroughly by pipetting.

8. Place an HE Column in a new HE Collection Tube.

9. Transfer 1000 µl of 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 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 ×g 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 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 of Wash Buffer 1 to the HE Column. Centrifuge for 1 min then discard flow-through.

13. Add 900 µl of Wash Buffer 2 (ethanol contained) to the HE Column. Centrifuge for 1 min then discard flow-through.

14. Add 500 µl of Wash Buffer 2 (ethanol contained) to the HE Column. Centrifuge for 2 mins to dry the membrane directly. Discard flow-through and the HE Collection Tube.

**15. OPTIONAL** for Marine Plants: Add 500 µl of Ethanol (96-100%) to HE Column centrifuge for 2 min. and discard the flow-through and stand the HE-Column for 5 min.

16. Place the HE Column in an Elution Tube, then add 30 µl of 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.

17. Centrifuge for 2 mins to elute RNA.

**For Research Use Only**