HiPure Plant RNA Plus Kit

Introduction

This product is suitable for extracting total RNA from 50-150mg conventional plant or fungal samples as well as samples rich in polyphenolic and polysaccharides. This kit is based on silica gel column purification technology, and the extraction process does not require the use of toxic phenol chloroform extraction. The entire extraction process only takes 20-30 minutes. This kit adopts DNA filtration technology, which can efficiently filter and remove DNA. The obtained RNA can be directly used for experiments such as RT-PCR, Northern Blot, poly A+ purification, nucleic acid protection, and in vitro translation.

Kit Contents

Product Number	R415002	R415003
Number of Preps	50 preps	250 preps
HiPure RNA Mini Columns	50	250
gDNA Filter Columns	50	250
2ml Collection Tubes	100	500
TCEP (1M)	0.29 g	5 x 0.29 g
Buffer EP	1.0 ml	5.0 ml
Buffer PSL	50 ml	250 ml
Buffer RW1	50 ml	250 ml
Buffer RW2*	20 ml	2 x 50 ml
RNase Free Water	6 ml	30 ml

Storage and Stability

Except TCEP, the kit components can be stored dry at room temperature (15–25°C) and are stable for at least 18 months under these conditions. After receiving the product, it is recommended to store TCEP (dry powder) at -20-8°C. At low temperatures, Buffer PSL may form precipitates, dissolve it completely by 55°C water bath.

Preparation

- Add 4 times volume absolute ethanol to Buffer RW 2 and store at room temperature.
- Dissolution of TCEP: Take out TCEP (dry powder), add 0.85ml Buffer EP to each tube, mix upside down, and dissolve before use. The dissolved TCEP can be stored at 2-8℃ for 3 months and -20℃ for 6 months. TCEP is an efficient, odorless, mercaptan free reducing agent that can effectively inactivate nuclease and prevent polyphenol oxidation, and can replace 2-Mercaptoethanol to improve the integrity and yield of RNA.
- Before use, add 20μl TCEP or 50μl 2-Mercaptoethanol to every 1ml Buffer PSL in order to improve the lysis and antioxidant capacity, the mixed solution can be placed for 1 week at 2-8℃.

Protocol 1:

1. Grind plants or fungi into powder with liquid nitrogen, weigh 50-150mg powder into a 2.0ml centrifuge tube.

- Processing easy-grind plant samples (fruit/fresh leaves, etc.), directly transfer 50-150mg samples and 1.0ml Buffer PSL to a mortar or electric homogenizer for grinding, and then transfer 0.8-0.9ml homogenization solution to a centrifuge tube, process follow the third step.
- Affected by the diversity of plant samples, and significant differences in tissue RNA content at different growth and development stages, during the initial experiment it is recommended to use sample amount of 100mg for plant or polyphenol samples, and 50mg for tissue samples rich in mucus. The sample amount can be adjusted based on the results, but it is not recommended to exceed 150mg.
- Grinded samples should not be thawed before weighing, transferring, storing, or adding PSL, otherwise RNA will degrade. After adding Buffer PSL, vortex for 10-15 seconds immediately to fully disperse the sample. The clustered sample can be dispersed by pipetting, and then grind the second sample.

2. Sample lysis: Add 0.9ml Buffer PSL to the sample and immediately vortex for 10-15 seconds to fully disperse the sample.

Before use, add 20μl TCEP or 50μl 2-Mercaptoethanol to every 1ml Buffer PSL in order to improve

the lysis and antioxidant capacity, the mixed solution can be placed for 1 week at 2-8℃.

3. Centrifuge at 14,000 x g for 5 minutes at room temperature.

4. Place gDNA Filter Column in a 2ml collection tube. Transfer ~750µl supernatant to a gDNA filter column. Centrifuge at 14,000 x g for 2 minutes and discard the gDNA filter column.

5. Add 0.4 times volume absolute ethanol (~300µl) to the filtrate and pipetting 3-5 times.

6. Place the HiPure RNA Mini Column in a 2ml collection tube. Transfer half volume of the mixture into the column. Centrifuge at 12,000 x g for 1 minute.

7. Discard the filtrate; insert the column back into the collection tube. Transfer the remain half of the mixture into the column. Centrifuge at 12,000 x g for 1 minute.

- If complete removal of DNA is required, it is recommended to order Dnase Set (C12133) for DNase digestion on membrane, following the steps of DNase digestion.

8. Discard the filtrate and insert the column into the collection tube. Add 700µl Buffer RW1 to the column. Centrifuge at 12,000 x g for 1 minute.

9. Discard the filtrate and insert the column into the collection tube. Add 700µl Buffer RW2 (diluted with ethanol) to the column. Centrifuge at 12,000 x g for 1 minute.
Buffer RW2 must be diluted with absolute ethanol. Dilute according to the bottle label or instructions.

10. Repeat step 9 once.

11. Discard the filtrate and insert the column into the collection tube. Centrifuge at 12,000 x g for 2 minutes.

12. Insert the column into a 1.5ml centrifuge tube. Add 30-100µl RNase Free Water to the membrane center of the column. Place at room temperature for 2 minutes. Centrifuge at 12,000 x g for 1 minute.

- The minimum elution volume of the column is 30µl. If RNA yield exceeds 30µg, it is recommended to elute a second time.

14. Discard the RNA binding column and store RNA at -80°C.

Optional Protocol: DNase digestion on membrane (additional order C12133 Dnase Set)

1. Follow steps 1-7 in Protocol 1 to bind RNA to HiPure RNA Mini Column.

2. **Discard the filtrate and insert the column into the collection tube. Add 300µl Buffer RW1 to the column.** Centrifuge at 12,000 x g for 2 minutes. Discard the filtrate and insert the column into the collection tube.

3. Prepare DNase I solution in 1.5ml centrifuge tube: 80µl Dnase and 10µl DNase I, mix well.

4. Add DNase I solution to the membrane center of the RNA binding column. Incubate at room temperature (15-30°C) for 15-20 minutes to remove DNA.

5. **Add 600µl Buffer RW1 to the column. Place for 2 minutes.** Centrifuge at 12,000 x g for 1 minute.

6. **Discard the filtrate and insert the column into the collection tube. Add 600µl Buffer RW2 (diluted with ethanol) to the column** and centrifuge 12,000 x g for 1 minute.

7. **Repeat step 6 once.**

8. Discard the filtrate and insert the column into the collection tube. Centrifuge at 12,000 x g for 2 minutes.

9. Insert the column into a 1.5ml centrifuge tube. **Add 30µl~80µl RNase Free Water to the membrane center of the column.** Place for 1 minute at room temperature. Centrifuge at 12,000 x g for 1 minute.

- The minimum elution volume of the column is 30µl. If RNA yield exceeds 30µg, it is recommended to elute a second time.
- This product only recover RNA >200nt. RNAs <200nt (approximately 15-20%), including 5S RNA and tRNA, are removed during the purification process which enrich mRNA.

10. Discard the column and store the RNA at -80 ° C.