HiPure HP Plant RNA Mini Kit

Introduction

This product is suitable for extracting high-purity total RNA (excluding miRNA) from plant samples which are difficult to extract (such as fruits and seeds). The kit combines two efficient RNA extraction technologies, the one-step RNA extraction technology and silica gel column RNA purification technology, to maximize the purity of RNA. 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	D416501	D416502	D4165-03
Preps per Kit	10 Preps	50 Preps	250 Preps
HiPure RNA Mini Columns	10	50	250
2ml Collection Tubes	10	50	250
DNase I	120 µl	600 µl	5 x 600 µl
DNase Buffer	1 ml	6 ml	30 ml
Buffer PAL	12 ml	60 ml	270ml
Buffer GXP2*	6 ml	20 ml	100ml
Buffer BDP	12 ml	60 ml	270ml
Buffer RW1	10 ml	50 ml	250ml
Buffer RW2*	5 ml	20 ml	2 x 50 ml
RNase Free Water	1.8 ml	10 ml	30ml

Storage and stability

DNase I should be stored at 2–8°C upon arrival. However, short-term storage (up to 12 weeks) at room temperature (15–25°C) does not affect their performance. The remaining kit components can be stored dry at room temperature (15–25°C) and are stable for at least 18 months under these conditions. The entire kit can be stored at 2–8°C, but in this case buffers should be redissolved before use. Make sure that all buffers are at room temperature when used.

Preparation

- Add 4 times volume absolute ethanol to Buffer RW2 and store at room temperature
- Add 1.5 times volume absolute ethanol to Buffer GXP2 and store at room temperature
- (Optional) 2-mercaptoethanol
- (Optional) PVP-40

Protocol A

This protocol is suitable for extracting RNA from various plant/fungal samples, especially complex and difficult-to-extract plant samples (such as grape leaves/tea).

1. Grind plants or fungi into powder with liquid nitrogen, add 50-100mg powder into a 2.0ml centrifuge tube.

2. **Immediately add 0.7ml Buffer PAL/2-mercaptoethanol to the sample, vigorously vortex to disperse the sample and place at 65℃ for 10 minutes.**

- Process easy-ground plant samples (fruit fruits/fresh leaves, etc.), transfer 100-150mg sample to a mortar, add 1ml Buffer PAL/2-mercaptoethanol to the mortar, immediately grind thoroughly, and then transfer 0.7ml homogenization solution to a 2.0ml centrifuge tube, place at 65℃ for 10 minutes, and follow step 3. (Optional) Before using Buffer PAL, add 2-mercaptoethanol to 1% (V/V).
- Process complex polyphenol samples, PVP-40 can be added to Buffer PAL before use, with a final concentration of 2% (W/V) to enhance the antioxidant capacity of the lysis buffer.

- Most samples do not require the addition of PVP-40 and mercaptoethanol. Due to the instability of 2-mercaptoethanol and PVP-40, after added Buffer PAL should not be stored at room temperature for more than one week. If downstream applications are extremely sensitive to DNA contamination, it is recommended that the sample amount not exceed 50mg. A large amount of DNA can cause incomplete digestion of DNase I.

3. **Add 700μl Buffer BDP into the lysis solution, vortex at high speed for 15 seconds.** Centrifuge at 13000 x g at room temperature for 5 minutes.

4. **Transfer ~500μl supernatant to a new centrifuge tube, add 1.5 times volume Buffer GXP2 to the supernatant,** and vortex for 15 seconds

- After adding Buffer GXP2, a small amount of flocculent precipitate is generated. Use a pipette to repeatedly aspirate and beat several times to try to disperse the flocculent matter. If a large amount of flocculent material is generated, it may be due to the presence of polysaccharides in the sample. When re-extracting, the sample size should be reduced by half or more to avoid clogging the column and improve the yield.

5. **Insert the HiPure RNA Mini Column into a 2ml collection tube. Transfer half volume the mixture (including precipitation) to the column.** Centrifuge at 12000 x g for 30-60 seconds.

6. Discard the filtrate, insert the column back into the collection tube, and **transfer the remaining mixed liquid (including precipitation) to the column**. Centrifuge at 12000 x g for 30-60 seconds.

7. Discard the filtrate, insert the column back into the collection tube. **Add 300μl Buffer RW1 to the column.** Centrifuge at 12000 x g for 60 seconds.

8. Discard the filtrate, insert the column back into the collection tube. **Prepare DNase I solution according to the following table and mix well. Add DNase I solution to the center of the RNA column membrane and place at room temperature for 20-30 minutes to remove DNA.**

Content	Amount
DNase I	10μl
DNase Buffer	100μl

9. Add 500μl Buffer RW1 into the column, place at room temperature for 3 minutes, centrifuge at 12000 x g for 30-60 seconds.

- (Optional) When processing plant samples rich in DNA, steps 8-9 can be repeated once to completely remove DNA.

10. Discard the filtrate, insert the column back into the collection tube. Add 500μl Buffer RW2 (Ethanol added) to the column. Centrifuge at 12000 x g for 30-60 seconds.

11. Discard the filtrate, insert the column back into the collection tube. Add 500μl Buffer RW2 (Ethanol added) to the column. Centrifuge at 12000 x g for 30-60 seconds.

12. Discard the filtrate, insert the column back into the collection tube. Centrifuge at 12000 x g for 2 minutes.

13. Transfer the column to a 1.5ml centrifuge tube, add 30-100μl RNase Free Water to the center of the column membrane. Place at room temperature for 2 minutes. Centrifuge at 12000 x g for 1 minutes.

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

Protocol B

This protocol uses a mild lysis solution (PlantZol Reagent, to be ordered separately), which is suitable for extracting RNA from fruit samples.

1. Plants and fungi: Grind plant or fungal samples into powder with liquid nitrogen, add 50-150mg powder into a centrifuge tube, immediately add 1ml PlantZol Reagent, vortex to disperse the sample, and place at room temperature for 5 minutes.

Process easily ground plant samples (fruit/fresh leaves, etc.), transfer 100-200mg samples to a mortar. Add 1.5ml PlantZol Reagent, immediately grind thoroughly, and then transfer 1ml homogenate into a 1.5ml centrifuge tube.

2. Add 200μl chloroform to the lysis solution, vortex for 15 seconds, and place at room temperature for 3 minutes.

3. Centrifuge at 13000 x g for 5 minutes at 2-8℃, operate following steps 4-14 of Protocol A.