

HiPure HP Plant RNA Mini Kit

Introduction

This product is suitable for extracting high-purity total RNA (including 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	R416501	R416502	R416503
Preps per Kit	20 Preps	50 Preps	250 Preps
HiPure RNA Mini Columns	20	50	250
2ml Collection Tubes	20	50	250
DNase I	600 µl	600 µl	5 x 600 µl
DNase Buffer	1 ml	6 ml	30 ml
Buffer PAL	12 ml	60 ml	270ml
Buffer BDP	12 ml	60 ml	270ml
Buffer GXP2*	6 ml	20 ml	100ml
Buffer RVC*	5 ml	20 ml	75ml
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 -20°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 24 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 2 times volume absolute ethanol to Buffer RWC 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

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°C for 10 minutes.**
- 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 700 µl Buffer BDP (or chloroform) 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 or 1.0 time volume of isopropanol (for miRNA Isolation) to the supernatant, and vortex for 15 seconds.**

After adding Buffer GXP2 or isopropanol, 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 RWC 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 minutes to remove DNA.**

Content	Amount
DNase I	10µl
DNase Buffer	100µl

9. Add 500µl Buffer RWC into the column, place at room temperature for 2 minutes, centrifuge at 12000 x g for 30-60 seconds.
10. Transfer the filtrate to column, and insert the column back into the collection Tube, 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. **Add 500µl Buffer RW2 (Ethanol added) to the column.** Centrifuge at 12000 x g for 30-60 seconds.
13. Discard the filtrate, insert the column back into the collection tube. Centrifuge at 12000 x g for 2 minutes.
14. **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.
15. Discard the column and store the RNA at -80 ° C.