HiPure Environmental DNA/RNA Kit

Introduction

This kit uses bead grinding method and silica gel column purification technology, which can quickly and safely extract RNA and DNA from soil samples, stool samples, environmental samples, and water samples simultaneously. The entire extraction process only takes 50 minutes. It is suitable to extract RNA and DNA separately from ⩽500mg soil samples, ⩽200mg stool samples, water filtration membranes, sediment or residue from fermentation liquid. The purified RNA can be directly used for experiments such as RT-PCR, Northern Blot, Poly A purification, nucleic acid protection, and in vitro translation. The purified DNA can be directly used for experiments such as PCR and Southern Blot.

Kit Contents

Product	R511701B	R511702B	R511703B
Preparation Times	10	50	250
HiPure RNA Mini Columns	10	50	250
HiPure DNA Mini Columns	10	50	250
2ml Collection Tubes	20	100	500
2ml Beads Tubes	10	50	250
Buffer STL	10 ml	40 ml	180 ml
Buffer SL	3 ml	15 ml	60 ml
Buffer PCI	1.5 ml	7 ml	30 ml
Buffer GXP	10 ml	50 ml	200 ml
Buffer GWP	6 ml	30 ml	150 ml
Buffer RW1	6 ml	30 ml	150 ml
Buffer RW2*	10 ml	50 ml	3 x 50 ml
Nuclease Free Water	1.8ml	15 ml	50 ml

Storage and Stability

This Kit can be stored dry at room temperature (15–25°C) and are stable for at least 24 months under these conditions.

Materials and Equipment to be Supplied by User

- 100% ethanol
- Dilute Buffer RW2 with 40ml (10 Preps), 200ml (50 Preps) or 3 x 200ml (250 Preps) 100% ethanol and store at room temperature.

Protocol

1. **Add proper biology samples to 2ml Bead Tubes** (~0.5g soil, 0.1-0.2g stool, 0.3-0.5g environmental samples, 0.3ml fermentation suspension, 0.3ml microbial suspension or other samples).

2. **Add 600µl Buffer STL and 100µl Buffer PCI to the sample, and screw the lid tightly.**

   Before using dry environmental samples, a sieve can be used to remove leaves, stones, or small branches as much as possible. For very dry materials, if sample absorbs too much lysis buffer, control the sample amount and increase the amount of Buffer STL appropriately. For very humid materials, it can centrifuge to remove excess liquid before adding lysis buffer. Control the sample amount to ensure sufficient space in the centrifuge tube for bead grinding. The reduction of starting materials usually helps to improve lysis efficiency and increase RNA purity.

3. Vortex at maximum speed for 10 minutes or place on a bead grinding machine for fast grinding with 30~90 seconds.
   - PowerLyzer grinder: recommend 2000rpm for 30s, pause for 30s and then repeat once.
   - FastPrep 24 grinder: recommend 5m/s for 30s, pause for 30s, and then repeat once.
   - Tissue Lysis II grinder: recommend 25Hz for 5min, reposition and then repeat once.

4. Centrifuge at 12,000 x g for 5 minutes at room temperature.

5. **Transfer the supernatant (~550µl) to a new centrifuge tube, add 200µl Buffer SL, vortex and mix for 5 seconds.** Centrifuge at 12,000 x g for 5 minutes.

6. **Transfer the supernatant to a new centrifuge tube, add 700µl binding solution Buffer GXP,** and invert 6-8 times to mix.

DNA Purification:

7. **Install the HiPure DNA Mini Column into a 2ml collection tube and transfer half volume of the mixture to the column**. Centrifuge at 10,000 × g for 1 minute and transfer the filtrate to a 5ml centrifuge tube.

8. **Install the DNA column back into the collection tube, transfer the remaining mixture to the column**, and centrifuge at 10,000 x g for 1 minute. Transfer the filtrate to the 5ml centrifuge tube (step 7). **Follow RNA Purification process from step 16.**

9. **Install the DNA column back into the collection tube, add 500µl Buffer GWP to the column**, and centrifuge at 10,000 x g for 1 minute.

10. **Discard the filtrate, install the column back into the collection tube, add 500µl Buffer RW2 to the column**. Centrifuge at 12,000 × g for 1 minute.

11. Repeat Step 10 once.

12. Discard the filtrate, install the column back into the collection tube. Centrifuge at 12,000 x g for 2 minute.

13. **Install the column in a new 1.5ml centrifuge tube. Add 30-50µl Nuclease Free Water (preheated to 65°C) directly to the center of the column membrane.** Place at room temperature for 5 minutes, then centrifuge at 12,000 × g for 1 minute.

14. **Add 30-50µl Nuclease Free Water (preheated to 65°C) directly to the center of the column membrane again.** Place at room temperature for 5 minutes. Centrifuge at 12,000 × g for 1 minute.

15. Discard the column and store the DNA at 2-8°C or -20°C.

RNA Purification

16. **Add 1/2 volume of 100% ethanol (100% ethanol: sample volume=1:2) to the sample (5ml centrifuge tube from step 8)**. Invert and mix 6-8 times to mix.

17. **Install HiPure RNA Mini Column into a 2ml collection tube. Transfer sample mixture (≤ 700µl) into the column.** Centrifuge at 12,000 x g for 1 minute.

18. **Discard the filtrate and install the column back into the collection tube. Transfer the remaining mixture to the column.** Centrifuge at 12,000 x g for 1 minute. Repeat this step until all the mixture is transferred to the column and centrifuged.

19. **Discard the filtrate, install the column back into the collection tube, add 500µl Buffer RW1 to the column.** Centrifuge at 12,000 × g for 1 minute.

20. **Discard the filtrate, install the column back into the collection tube, add 500µl Buffer RW2 to the column.** Centrifuge at 12,000 × g for 1 minute.

21. Repeat Step 20 once.

22. Discard the filtrate, install the column back into the collection tube. Centrifuge at 12,000 x g for 2 minute.

23. **Transfer the column to a new 1.5ml centrifuge tube, add 30-100µl Nuclease Free Water directly to the center of the column membrane**. Place at room temperature for 2 minutes. Centrifuge at 12,000 × g for 1 minute.

24. Discard the column and store the RNA at -20°C or -80°C.