HiPure Stool RNA Kit

Introduction

HiPure Stool RNA Kit is specially designed for stool RNA extraction. The kit is suitable for extracting high-purity microbial or host cell RNA from ≤0.1g stool samples. This kit adopts silica gel column purification technology and an innovative solution system, which can efficiently remove inhibitory factors such as humic acid from stool samples. The purified RNA can be directly used in RT-PCR and Northern hybridization.

Principle

The HiPure silica gel column uses a high binding ability glass fiber filter membrane as the substrate. Under the condition of high concentration of ionizing agent (such as Guanidinium chloride or guanidine isothiocyanate), the filter membrane can adsorb nucleic acid through hydrogen bonding and electrostatic and other physical factors, while protein or other impurities are not adsorbed and removed. The filter membrane that has adsorbed nucleic acids is washed to remove proteins and salts. Finally, low salt buffer solution (such as Buffer TE) or water can be used to wash out the nucleic acids adsorbed on the filter membrane. The obtained nucleic acid has high purity and can be directly used in various downstream experiments.

The stool samples are homogenized in the lysis solution, further lysed in a high-temperature water bath, and RNA is released into the lysis solution. Chloroform extraction removes genomic DNA and impurities, transfers the supernatant to an alcohol free binding solution, purifies RNA through a column, and finally elutes RNA with RNase Free Water. The purified RNA can be directly used for experiments such as PCR, Southern hybridization, and enzyme digestion.

Storage and Stability

The kit components can be stored at room temperature (15–25°C) and are stable for 18 months under these conditions. At low temperatures, Buffer SPL may form precipitates, dissolve it by 55°C water bath. After receiving the product, Buffer PHC should be stored at 2-8°C.

Kit Contents

Product Number	R418502	R418503
Purification Times	50 preps	250 preps
HiPure RNA Mini Columns	50	250
2ml Collection Tubes	50	250
Glass Beads (0.1~0.6mm)	30 g	150 g
Buffer SPL	30 ml	140 ml
Buffer PHC	30 ml	140 ml
Buffer GRP	60 ml	250 ml
Buffer RW1	50 ml	250 ml
Buffer RW2*	20 ml	2 x 50 ml
RNase Free Water	15 ml	30 ml

Materials and Equipment to be Supplied by User

- Absolute ethanol (96-100%)
- 1.5ml centrifuge tube without RNase enzyme and pipette tip without RNase enzyme
- (Optional) 65°C water bath pot
- Microcentrifuge capable of at least 12,000 × g
- Chloroform
- Add 80ml (50 Preps) or 200ml (250 Preps) 100% ethanol to the bottle of RW2 and stored at room temperature

Protocol 1:

1. Add ~0.5g glass beads (0.1-0.6mm) to a 2ml centrifuge tube.

2. Take 100-150mg stool samples into a centrifuge tube containing glass beads. If the sample is liquid, pipette 0.1ml sample into a 2ml centrifuge tube. Cut off the head of the 1ml pipette tip for easy transfer.

When processing animal stool samples rich in fiber (such as cattle and sheep), sample amount is 50-100mg. When processing animal stool with minimal water content (such as mouse), sample amount is 30-60mg.

3. Immediately add 0.5ml Buffer SPL and 0.5ml PHC to the sample, and vortex for up to 10 minutes to disperse the sample. 65°C water bath for 15 minutes, during which vortex for 5 seconds every 1 minute to mix well.

Omitting this step can reduce the interference of bacterial RNA when only extracting host cell RNA.

4. Add 0.5ml chloroform to the lysate solution and vortex for 15 seconds. Incubate at room temperature for 5-10 minutes.

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

6. Carefully transfer the supernatant into a new centrifuge tube. Add twice volume Buffer GRP to the supernatant. Vortex for 10 seconds.

7. Insert the HiPure RNA Column into a 2ml collection tube. Transfer half of the mixture into the column. Centrifuge at 10,000 x g for 30-60 seconds.

8. Discard the filtrate and insert the column into the collection tube. Transfer the remaining mixture to the column. Centrifuge at 10,000 x g for 30-60 seconds.

9. Discard the filtrate and insert the column into the collection tube. Add 500μl Buffer RW1 to the column. Centrifuge at 10,000 x g for 1 minute.

10. Discard the filtrate and insert the column into the collection tube. Add 500μl Buffer RW2 (diluted with ethanol) to the column. Centrifuge at 10,000 x g for 1 minute.

Buffer RW2 must be diluted with absolute ethanol. Dilute according to the bottle label or instructions.

11. Repeat Step 10 once.

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

13. Insert the column into a 1.5ml centrifuge tube. Add 30-50μl RNase Free Water to the membrane center of the column. Place for 1 minute. Centrifuge at 12,000 x g for 1 minute.

14. Discard the RNA binding column and store at -20℃ or -80℃ .

Optional Protocol: DNase digestion on membrane

Although HiPure Stool RNA Kit can effectively remove genomic DNA contamination, for

sensitive downstream applications such as RT-PCR, trace amounts of DNA contamination can cause significant interference. Thoroughly removing DNA contamination can be achieved through DNase digestion. Additional purchase of **Dnase Set (C12133)** is required.

1. According to the sample type, follow the step 1-8 in Protocol 1 to homogenize and adsorb RNA on the column.

2. Discard the filtrate and insert the column into the collection tube. **Add 400µl Buffer RW1 to the column.** Centrifuge at 10,000 x g for 1 minute.

3. Discard the filtrate and insert the column into the collection tube. Prepare DNase I solution according to the following table and gently mix well.

(Note: When removing the RNA column, do not let the bottom of the column contact with the solution)

Content	Amount
DNase Buffer	60 µl
DNase I(10Units/µl)	10 µl

4. Add DNAse I solution to the membrane center of the RNA binding column. Incubate at 25-37°C for 15-20 minutes. DNase I solution should be added to the center of the column membrane, not to the wall.

5. **Add 500µl Buffer RW1 to the column.** Place for 2 minutes. Centrifuge at 10000 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 10000 x g for 1 minute.

Buffer RW2 must be diluted with absolute ethanol. Dilute according to the bottle label or instructions.

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 to dry the column.

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

10. Discard the column and store the RNA at -20℃ or -80℃.