# HiPure Soil RNA Kit

## Introduction

The HiPure SoilRNA Kit is suitable for extracting high purity total RNA from Soil or other environmental samples The kit combines the SDS/Phenol and silica gel column purification technology to complete the extraction of high purity total RNA in only 40 minutes.The obtained RNA can be directly used for RT-PCR, Northern blot, Poly-A + purification, nucleic acid protection and in vitro translation experiments.

## Kit Contents

| Product Number | R418301 | R418302 | R418303 |
|---|---|---|---|
| Purification Times | 10 preps | 50 preps | 250 preps |
| HiPure RNA Mini Columns I | 10 | 50 | 250 |
| gDNA Filter Column | 10 | 50 | 250 |
| 2ml Collection Tubes | 20 | 100 | 500 |
| 2ml Beads Tubes | 10 | 50 | 250 |
| Buffer SOL | 6 ml | 30 ml | 150 ml |
| Buffer SDS | 1 ml | 4 ml | 15 ml |
| Buffer PHC | 6 ml | 30 ml | 150 ml |
| Buffer GDP | 10 ml | 40 ml | 150 ml |
| Buffer RW1 | 10 ml | 50 ml | 200 ml |
| Buffer RW2 * | 5 ml | 20 ml | 2 x 50 ml |
| RNase Free Water | 1.8 ml | 10 ml | 30 ml |

## Storage and Stability

Buffer PHC 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.

## Materials and Equipment to be Supplied by User

- absolute ethyl alcohol(96-100%)
- chloroform
- Buffer RW2 was diluted with anhydrous ethanol and stored at room temperature

## 【Protocol】

1. Transfer 500mg soil, 50-100mg Stool or 200-500mg other environmental samples to 2ml Bead Tubes.
2. Add 500μL Buffer SOL, 50μL Buffer SDS and 500μL Buffer PHC to the sample. Lyse sample by vortex at maximum speed for 10 minutes or by Fastpreps 24 ( 6.5 m/s twice for 45s).

   For best results, a mixer mill, such as GenoGrinder 2010, Fastprep-24®, or Omni Bead Ruptor should be used.
3. Centrifuge for 5 seconds to remove drops of liquid from the lid.
4. Add 200μL chloroform and vortex to mix thoroughly. Incubate for 3 minutes.
5. Centrifuge at maximum speed (≥13,000 x g) for 10 min at 4°C.
6. Transfer the cleared supernatant (~400μL) to a new 2.0 mL microcentrifuge tube.

7. Add an equal volume Buffer GDP and mix by inverting the tube 4-6 times.

8. Insert a gDNA Filter Column into a 2.0mL Collection Tube (provided).

9. Transfer the Mixture from step 7 to a the DNA column placed in a 2 ml collection tube (supplied). Centrifuge for 60 s at ⩾12000 x g . Discard the column, and save the flow-through.

10. Add 0.5 volume of absolute ethanol to the flow-through, and mix well by pipetting.

11. Insert a HiPure RNA Mini Column in a 2ml Collection Tube.

12. Add up to 700µl of the sample from Step 10 to the Column. Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.

13. Repeat Step 12 until all of the sample has been transferred to the column.

14. Add 700µl Buffer RW1 to the column, centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.

15. Add 500µl Buffer RW2 to the column, Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.

16. Add 500µl Buffer RW2 to the column, Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.

17. Centrifuge the empty Column at 12,000 × g for 2 minute at room temperature to dry the column matrix.

18. Transfer the Column to a clean 1.5ml microcentrifuge tube. Add 25~50µl RNase Free Water directly to the center of the column membrane. Let sit at room temperature for 2 minutes.

19. Centrifuge at 12,000 × g for 1 minute at room temperature. Store RNA at -20℃.

    If the expected RNA yield is >30 µg, repeat step 10 using another 30–50µl RNase-free water, or using the eluate from step 12-13.

## Troubleshooting Guide

### 1. Clogged HiPure RNA Column

- **Too much starting material:** In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material.
- **Inefficient disruption and/or homogenization:** Disrupting and homogenizing starting materia as RNeasy Mini Kit pages 18-21.

### 2. RNA does not perform well (e.g. in RT-PCR

- **Salt concentration in eluate too high**: Modify the wash step by incubating the column for 5 min at room temperature after adding 500ul of Buffer RW2, then centriufge.
- **Eluate contains residual ethanol:** Ensure that the wash flow-through is drained from the collection tube and that the column is then centrifuged at >12,000 x g for 1min.

### 3. DNA contamination in downstream expeiments

- **No DNase treatment**: Perform optional on column DNase digestion using RNase-Free DNase Ste at the point individual protocols.