HiPure Fibrous DNA/RNA Kit

Introduction

The Kit is designed to purify genomic DNA and total RNA simultaneously from a single biological sample. Lysate is first passed through an DNA spin column to selectively isolate DNA and then through an RNA column to selectively isolate RNA. Pure DNA and RNA are purified from the entire sample, in contrast to other procedures where either the biological sample or the purified total nucleic acids is divided into two before being processed separately. The kit is compatible with small amounts of a wide range of animal cells and tissues.

Principle

The Kits are designed to purify both genomic DNA and total RNA from the same cell or tissue sample. Samples are first lysed and homogenized. The lysate is passed through a DNA Mini column and bind DNA. Ethanol is added to the flow-through and the sample is applied to an RNA column. DNA/RNA binds to the membrane and contaminants are washed away. High-quality RNA is eluted in as little as 30 µl water using the Kit. High-quality dNA is eluted in as little as 50 µl water using the Kit.

Kit Contents

Product	R511402	R511403
Preparation Times	50	250
HiPure DNA Mini Column	50	250
HiPure RNA Mini Columns	50	250
2ml Collection Tubes	100	2 x 250
Buffer RL	30 ml	150 ml
RNA Digestion Buffer	15 ml	80 ml
Buffer DW1	30 ml	150 ml
Buffer RW1	50 ml	200 ml
Buffer RW2*	20 ml	2 x 50 ml
RNase Free Water	10 ml	30 ml
Buffer AE	10 ml	50 ml

Storage and Stability

HiPure DNA/RNA Kit can be stored dry at room temperature (15–25°C) and are stable for at least 18 months under these condition. s Make sure that all buffers are at room temperature when used. During shipment, crystals or precipitation may form in the Buffer RLC. Dissolve by warming buffer to 37°C.

Materials and Equipment to be Supplied by User

- Dilute Buffer RW2 with 80ml (50 Preps) or 2x200ml (250 Preps) 100% ethanol and store at room temperature
- Microcentrifuge capable of at least 12,000 × g
- If purifying RNA from cell lines rich in RNases, we recommend adding β-mercaptoethanol (β-ME) to Buffer RLC before use. Add 10µl β-ME per 1ml Buffer RLC. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLC containing β-ME can be stored at room temperature (15–25°C) for up to 1 month. Alternatively, add 20µl of 2M dithiothreitol (DTT) per 1 ml Buffer RLC. The stock solution of 2 M DTT in water should be prepared fresh or frozen in single-use aliquots. Buffer RLC containing DTT can be stored at room temperature for up to 1 month.

Protocol:

1. **Do not use more than 20mg Animal Tissue. Disruption and homogenination of sample with 500µl Buffer RL.**
2. **Add 250µl RNA Digestion Buffer to the sample and mix well.**
3. Add 20µl Proteinase K to the sample and mix well. Incubate at 55°C for 15 minutes. Centrifuge at 13,000 x g for 5 min.
4. Insert a HiPure DNA Mini Column in a 2ml Collection Tube.
5. **Transfer the supernatant to a the DNA Mini column. Centrifuge for 60 s at ⩾12000 x g.**
6. Place the HiPure DNA Mini column in a new 2 ml collection tube (supplied) , and store at room temperature for later DNA purification in steps 17–21. Use the flow-through for RNA purification in steps 7–16.

Total RNA Isolation

7. **Add 0.5 volume of absolute ethanol to the flow-through, and mix well by pipetting.** Do not centrifuge.

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

9. **Add up to 700µl of the sample from Step 7 to the Column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.

10. Repeat Step 9 until all of the sample has been transferred to the column.

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

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

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

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

15. **Transfer the column to a clean 1.5ml microcentrifuge tube. Add 30~100µl RNase Free Water directly to the center of the column membrane.** Let sit at room temperature for 2 minutes.

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

Genomic DNA Isolation:

17. **Add 500µl Buffer DW1 to the DNA Mini column from step 6 and incubate for 2min. Centrifuge at 12,000 × g for 1 minute.** Discard the filtrate and reuse collection tube.

18. **Add 650µl Buffer RW2 to the column, Centrifuge at 12,000 × g for 1 minute.** Discard the filtrate and reuse collection tube.

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

20. **Transfer the Column to a clean 1.5ml microcentrifuge tube. Add 50-100µl Buffer AE to the**

center of the column membrane. Let sit at room temperature for 3 minutes. Centrifuge at 12,000 × g for 1 minute at room temperature.

21. **For maximum concentration/Yield: Reload the eluate containing the DNA or pipet a new 50~100µl Buffer AE onto the membrane of th column.** Close the cap and incubate at room temperature for 5 min.

22. Centrifuge at 12,000 x g for 1 min. Store DNA at -20℃.

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.