HiPure 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	R511102	R511103
Preparation Times	50	250
HiPure DNA Mini Column	50	250
HiPure RNA Mini Columns	50	250
2ml Collection Tubes	100	2 x 250
Buffer RLC	50 ml	200 ml
Buffer DW1	30 ml	150 ml
Buffer RW1	30 ml	150 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 conditions.

Materials and Equipment to be Supplied by User

- Dilute Buffer RW2 with 80ml (50 Preps) or 2 x 200ml (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

1A. Cell: **Harvest cells no more than 1 x 10^7 cells.**

For pelleted cells: loosen the cell pellet thoroughly by flicking and add 700µl of Buffer RLC. For direct lysis of cells grown in a monolayer: Add 750µl of Buffer RLC to the cell-culture dish. Collect the lysate with a rubber policeman and pipet it into a microcentrifuge tube. **Pass the lysate at least 5 times through a blunt 20-gauge needle fitted to an RNase-Free Sringe.** Proceed to step 2.

1B. Tissue: **Do not use more than 30mg Animal Tissue.**

Disruption and **homogenination of sample with 750µl Buffer RLC.** After lysate, centrifuge at 14,000 x g for 3 minute at room temperature. Proceed to step 2.

RNA yields from skeletal muscle, heart and skin tissue may be low due to the abundance of contractile proteins, connective tissue and collagen. For purification of genomic DNA and total RNA from these tissues, we recommend using theHiPure Fibrous DNA/RNA Kit.

1C: Plant Tissue:

Disruption Plant sample by liquid nitrogen, Transfer up to 100mg power to 1.5ml microcentrifuge Tube, then add 750µl Buffer RLC and mix well by vortexing. Centrifuge at 14,000 x g for 3 minute at room temperature. Proceed to step 2.

2. Insert a HiPure DNA Mini Column in a 2ml Collection Tube.

3. Transfer the homogenized lysate or supernatant to a the DNA Mini column. Centrifuge for 60 s at ⩾12000 x g.
   Make sure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.

4. **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 15–19. Use the flow-through for RNA purification in steps 5–14.**

5. **Add 1 volume (usually 700µl) of 70% ethanol to the flow-through, and mix well by pipetting.** Do not centrifuge.
   If some lysate was lost during homogenization and DNA removal, adjust the volume of ethanol accordingly. For maximum RNA yields from liver, use 50% ethanol instead of 70% ethanol.

Total RNA Isolation

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

7. **Add up to 700µl of the sample from Step 5 to the Column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.

8. Repeat Step 7 until all of the sample has been transferred to the column.

9. **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.

10. **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.

11. **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.

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

13. **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.

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

Genomic DNA Isolation:

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

16. **Add 650µl Buffer RW2 to the column, Centrifuge at 12,000 × g for 1 minute.** 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. Place the DNA spin column in a new 1.5 ml collection tube (supplied). **Add 50 µl Buffer EB directly to the spin column membrane and close the lid.** Incubate at room temperature (15–25°C) for 2 min, and then centrifuge for 1 min at 12,000 x g to elute the DNA.

19. Repeat step 18 to elute further DNA.

    To prevent dilution of the first DNA eluate, use a new 1.5 ml collection tube (not supplied) to collect the second DNA eluate. To combine the first and second DNA eluates, reuse the collection tube from step 18.

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.