HiPure DNA/RNA/Protein Kit

Introduction

The Kit provides fast purification of high-quality DNA, RNA and Prote from cells, tissues, and yeast using silica-membrane spin columns with a binding capacity of 100ug RNA. There is no need for phenol/chloroform extractions and time-consuming steps such as CsCl gradient ultracentrifugation, or precipitation with isopropanol or LiCl are eliminated. RNA purified using the HiPure Total RNA Purification System is ready for applications such as RT-PCR, Northern blotting, poly A+ RNA (mRNA) purification, nuclease protection, and in vitro translation.

Principle

The Kit isolates total RNA from up to10^7 cells or 30 mg tissue. A short workflow enables RNA isolation with genomic DNA removal in less than 25 min. Samples are first lysed and homogenized. The lysate is passed through a DNA Mini column, ethanol is added to the flow-through, and the sample is applied to an RNA column. 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.

Kit Contents

Product	R521102	R521103
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 GW1*	22 ml	66 ml
Buffer RW1	50 ml	200 ml
Buffer RW2*	50 ml	3 x 50 ml
RNase Free Water	10 ml	30 ml
Elution Buffer	10 ml	30 ml
Buffer ALO(5%SDS)	10 ml	30 ml

Storage and Stability

HiPure Kit can be stored dry at room temperature (15–25°C) and are stable for at least 18 months under these conditions. 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

- Add 28ml (50 preps) or 84ml (250 preps) 100% ethanol to the bottle of Buffer GW1 and store at room temperature.
- Add 200ml (50 preps) or 3x300ml (250 preps) 100% ethanol to the bottle of RW2 and store at room temperature.
- 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. **Homogenization and lysis of samples.**

1A. Cell: **Harvest cells no more than 1 x 10^7 cells.** For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Add the appropriate volume of Buffer RLC. For direct lysis of cells grown in a monolayer, add the appropriate volume of Buffer RLC to the cell-culture dish. **Pass the lysate at least 5 times through a blunt 20-gauge needle fitted to an RNase-Free Sringe.** Transfer the lysate to a new clean 1.5ml Tube. Proceed to step 2.

- ≤5 x 10^6/6mm Dish: Add 500µl Buffer RLC;
- ≥5 x 10^6 / 6～10mm Dish:Add 700µl Buffer RLC;

1B. Animal Tissue : **Do not use more than 20 mg Animal Tissue. Disruption and homogenination of sample, then add Buffer RLC.** After lysate, centrifuge at 14,000 x g for 3 minute at room temperature. Transfer the cleared supernatant to a new clean 1.5ml Tube. Proceed to step 2.

- ≤10mg：Add 500µl Buffer RLC;
- >10mg：Add 700µl Buffer RLC;

1C: Plant Tissue: **Disruption Plant sample by liquid nitrogen, Transfer up to 150mg power to 1.5ml Tube, then add 700µl Buffer RLC and mix well by vortexing.**Centrifuge at 14,000 x g for 3 minute at room temperature. Transfer the cleared supernatant to a new clean 1.5ml Tube. 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 column placed in a 2 ml collection tube (supplied).** 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 (15–25°C) or at 4°C for later DNA purification in steps 13–18. Use the flow-through for RNA purification in steps 5–12.**
Note: Do not store the HiPure DNA Mini column at room temperature or at 4°C for long periods. Do not freeze the column.

Total RNA purification

5. **Add 0.5 volume (usually 250µl or 350µl) of absolute 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. When purifying RNA from certain cell lines, precipitates may be visible after addition of ethanol. This does not affect the procedure.

6. Insert a HiPure RNA Mini Column in a 2ml Collection Tube. **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.Transfer the flow- through* to a 2 ml tube for protein purification in steps 19–23. Reuse the collection tube in step 7.

7. If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same spin column. Transfer the flow-through after each centrifugation to the 2 ml tube.

8. **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.

9. **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.

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

12. **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. 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.

Genomic DNA purification

13. **Add 500 µl Buffer GW1 to the DNA column from step 4.** Close the lid gently, and centrifuge for 15 s at 8000 x g (10,000 rpm) to wash the spin column membrane. Discard the flow-through.

14. **Add 500 µl Buffer RW2 to the DNA spin column.** Close the lid gently, and centrifuge for 15 s at 8000 x g (10,000 rpm) to wash the spin column membrane. Discard the flow-through.

15. **Add 500 µl Buffer RW2 to the DNA spin column.** Close the lid gently, and centrifuge for 15 s at 8000 x g (10,000 rpm) to wash the spin column membrane. Discard the flow-through.

16. Centrifuge the empty Column at 8,000 × g for 2 minute at room temperature to dry the column matrix.

17. **Transfer the Column to a clean 1.5ml microcentrifuge tube. Add 50µl Elution Buffer (preheated to 70°C) directly to the center of the column membrane.** Let sit at room temperature for 5 minutes and then centrifuge for 1 min at 8000 x g (10,000 rpm) to elute the DNA.

18. Repeat step 17 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.

Total protein precipitation

19. **Add 4 volume of ice-cold acetone to the flow-through from step 6~7.** Mix vigorously and incubate at room temperature for 10 min to precipitate protein.

20. Centrifuge at full speed for 10 min, and carefully decant the supernatant.

21. Add 500 µl of 70% ethanol to the protein pellet. Centrifuge at full speed for 1 min, and remove the supernatant by using a pipet or by decanting as much liquid as possible. It is not necessary to resuspend or incubate the pellet.

22. **Dry the protein pellet for 5–10 min at room temperature.**

23. **Add up to 100 µl Buffer ALO and mix vigorously to dissolve the protein pellet.**
    Note: The volume of Buffer ALO to add depends on the amount of starting material. Buffer ALO is a Laemmli-related sample buffer for use in SDS-PAGE. If the proteins will not be analyzed by SDS-PAGE, dissolve the pellet in a buffer compatible with the intended downstream application. Due to the strong denaturing conditions with Buffer RLT, which is necessary to inactivate RNases and proteases, the precipitated proteins may show reduced solubility. Vortex for several minutes or disaggregate the pellet by pipetting up and down several times. Depending on the sample type, the pellet may contain proteins or other cellular components that are not soluble.