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HiPure Total RNA Plus Kit

Introduction

The Kit provides fast purification of high-quality RNA 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	R411102	R411103
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 RW1	50 ml	200 ml
Buffer RW2*	12 ml	2 x 50 ml
RNase Free Water	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

- Dilute Buffer RW2 with 48ml (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

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 350μl 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 400μl 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 . Discard the column, and save the flow-through.
   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. **Add 1 volume (usually 350μl or 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. When purifying RNA from certain cell lines, precipitates may be visible after addition of ethanol. This does not affect the procedure. For maximum RNA yields from liver, use 50% ethanol instead of 70% ethanol.
5. Insert a HiPure RNA Mini Column in a 2ml Collection Tube.
6. **Add up to 700μl of the sample from Step 4 to the Column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
7. Repeat Step 6 until all of the sample has been transferred to the column.
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.

13. 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.