HiPure Total RNA 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 System is ready for applications such as RT-PCR, Northern blotting, poly A+ RNA (mRNA) purification, nuclease protection, and in vitro translation.

Principle

HiPure RNA technology simplifies total RNA isolation. Samples are first lysed and then homogenized. Ethanol is added to the lysate to provide ideal binding conditions. The lysate is then loaded onto the HiPure silica membrane and RNA binds to the silica membrane, and all contaminants are efficiently washed away. For certain RNA applications that are sensitive to very small amounts of DNA, the residual amounts of DNA remaining can be removed using a convenient on-column DNase treatment. Pure, concentrated RNA is eluted in water.

Kit Contents

Product	R401100	R401102	R401103
Preparation Times	20	50	250
HiPure RNA Mini Columns	20	50	250
2ml Collection Tubes	20	50	250
RTL Lysis Buffer	15 ml	50 ml	200 ml
RNA Binding Buffer*	4.5 ml	15 ml	75 ml
Buffer RW1	15 ml	50 ml	200 ml
Buffer RW2*	6 ml	20 ml	2 x 50 ml
RNase Free Water	5 ml	10 ml	30 ml

Storage and Stability

The 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

- Add 24ml (20 Preps), 80ml (50 Preps) or 2 x 200ml (250 Preps) 100% ethanol to the bottle of RW2 and store at room temperature.
- Add 10.5 ml (20 Preps), 35 ml (50 Preps) or 175ml (250 Preps) 100% ethanol to the bottle of RNA Binding Buffe rand store at room temperature
- Microcentrifuge capable of at least 12,000 × g
- (Optional) 2-mercaptoethanol can be added to an aliquot of RTL Lysis Buffer before use. Add 20µl 2-mercaptoethanol per 1mL RTL Lysis Buffer. This mixture can be stored for 2 weeks at room temperature
- RNA yields from fibrous tissues, such as skeletal muscle, heart, and skin, may be low due to the abundance of contractile proteins, connective tissue, and collagen. For maximum RNA yields from these tissues, we recommend using the HiPure Fibrous Tissue RNA Kit instead.

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 RTL Lysis Buffer. For direct lysis of cells grown in a monolayer, add the appropriate volume of Buffer RLT 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 RTL Lysis Buffer；
- ≥5 x 10^6 / 6～10mm Dish:Add 700µl RTL Lysis Buffer；

1B. Animal Tissue : **Do not use more than 20 mg Animal Tissue. Disruption and homogenination of sample, then add RTL Lysis Buffer.** 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 RTL Lysis Buffer；
- >10mg：Add 700µl RTL Lysis Buffer；

1C: Plant Tissue: **Disruption Plant sample by liquid nitrogen, Transfer up to 150mg power to 1.5ml Tube, then add 700µl RTL Lysis Buffer 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.

1D: Yeast Cell: Collect 5 x 10^6 yeast cells, then add 300mg 0.4-0.6g Glass Beads and 400µl RTL Lysis Buffer. Vortex at maxi speed for 10min. Centrifuge at 10,000 x g for 3 minute at room temperature. Transfer the cleared supernatant to a new clean 1.5ml Tube. Proceed to step 2. (Glass Beads need to order separately).

1E: Bacterial Cell: Collect 1 x 10^8 bacterial cells, then add 300mg 0.1-0.26g Glass Beads and 400µl RTL Lysis Buffer. Vortex at maxi speed for 10min. Centrifuge at 10,000 x g for 3 minute at room temperature. Transfer the cleared supernatant to a new clean 1.5ml Tube. Proceed to step 2.

1F: RNA Clean up: Adjust the RNA Sample to a volume of 100µl with RNase Free Water. Add 300µl RTL Lysis Buffer and mix well. Add 300µl absolute ethanol and mix well. Proceed to step 3.

2. **Add 1 volume of RNA Binding Buffer (Dilute with 100% ethanol prior to use) to the lysate.** mix immediately by pipetting.
   When purifying RNA from certain cell lines, precipitates may be visible after addition of ethanol. This does not affect the procedure.

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

4. **Add up to 700µl of the sample from Step 2 to the Column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.

5. Repeat Step 4 until all of the sample has been transferred to the column.

6. **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.
   If performing optional on-column DNase digestion to completely remove DNA.

7. **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.

8. **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.

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

10. **Transfer the Column to a clean 1.5ml microcentrifuge tube. Add 30~50µl 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.

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

11. Store RNA at -80℃.

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 qiagen RNeasy Mini Kit pages 18-21. If working with tissues rich in proteins, we recommend using the HiPure Fibrous Tissue RNA Mini Kit.

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.
- **Incubation with Buffer RW1：** In subsequent preparations, incubate the RNeasy spin column for 5 min at room temperature after addition of Buffer RW1 and before centrifuging.

4. Low A260/A280 value

- Water used to dilute RNA for A260/A280 measurement: Use 10 mm Tris·Cl, pH 7.5, not RNAse-free water, to dilute the sample before measuring purity.