HiPure Cell miRNA Kit

Introduction

The Kit is designed for purification of total RNA, including miRNA and other small RNA molecules(18nt), from cultured cells and various animal and human tissues, including difficult-to-lyse tissues samples.

Principle

Biological samples are first lysed and homogenized in a highly denaturing guanidine isothiocyanate-containing buffer, which immediately inactivates DNases and RNases to ensure isolation of intact DNA and RNA. The lysate is then passed through an Mini spin column. This column, in combination with the high-salt buffer, allows selective and efficient binding of genomic DNA. Flow-through from the column is digested by Proteinase K in the presence of ethanol. This optimized digestion, together with the subsequent addition of further ethanol, allows appropriate binding of total RNA, including miRNA, to the column. Contaminants are efficiently washed away and high-quality RNA is eluted.

Kit Contents

Product	R431102	R431103	Contents
Preparation Times	50	250	—
HiPure RNA Mini Columns	100	2 x 250	Silica Column
2ml Collection Tubes	100	2 x 250	PP Column
Proteinase K	48 mg	240 mg	Proteinase K
Protease Dissolve Buffer	5 ml	15 ml	Tris/Glycerol/CaCl2
Buffer RLC	40 ml	200 ml	Guanidine Salt/Phenol
Buffer RWC*	20 ml	80 ml	Guanidine Salt
Buffer RW2*	20 ml	2 x 50 ml	Tris/EDTA
RNase Free Water	10 ml	60 ml	DEPC-Treated Water

Storage and Stability

Proteinase K should be stored at 2–8°C upon arrival. However, short-term storage (up to 12 weeks) at room temperature (15–25°C) does not affect their performance. The remaining kit components can be stored 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
- Dilute Buffer RWC with 40ml (50 Preps) or 160ml (250 Preps) 100% ethanol and store at room temperature
- Add 2.4ml (50Preps) or 12ml (250 Preps) Protease Dissolve Buffer to the Proteinase K and store at -20~8°C after dissolve.
- Microcentrifuge capable of at least 12,000 × g

Protocol

1. **Homogenization and lysis of samples.**

1A. Cell: **Harvest cells no more than 5 x 10^6 cells.** For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Add 500µl Buffer RLC. For direct lysis of cells grown in a monolayer, add 600µl 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.**

1B. Animal Tissue : **Do not use more than 10 mg Animal Tissue. Disruption and homogenination of sample, then add 600µl Buffer RLC.** After lysate, centrifuge at 14,000 x g for 3 minute at room temperature.

1C: Plant Tissue: **Disruption Plant sample by liquid nitrogen, Transfer up to 50mg power to 1.5ml Tube, then add 600µl Buffer RLC and mix well by vortexing.**Centrifuge at 14,000 x g for 3 minute at room temperature.

Isolation Total RNA (include miRNA)

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

3. **Transfer the homogenized lysate or supernatant to a the 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.

4. Transfer 450μl of the flow-through into a new 2 ml microcentrifuge tube.
5. Add 200 μl RNase Free Water and 250 μl absolute ethanol to the sample and mix well.
6. Add 40μl Proteinase K to the sample. Invert to mix well and incubate at room temperature for 15 min.
7. Add 750μl of absolute ethanol to the supernatant, and mix thoroughly by vortexing. Proceed step 8.

Enrichments of miRNA

2. Transfer 500μl the homogenized or supernatant into a new microcentrifuge tube. Add 150μl absolute ethanol to the sample and mix well.
3. Insert a HiPure RNA Mini Column in a 2ml Collection Tube.
4. Add the mixture from Step 2 to the RNA Column. Centrifuge at 10,000 × g for 1 minute at room temperature.
5. Transfer 650μl of the flow-through into a new 2 ml microcentrifuge tube.
6. Add 40μl Proteinase K and 150μl RNase Free Water to the sample. Invert to mix well and incubate at room temperature for 15min.
7. Add 850μl absolute ethanol to the sample and mix thoroughly by vortexing. Proceed step 8.
8. Insert a HiPure RNA Mini Column in a 2ml Collection Tube.
9. Add 750μl of the mixture from Step 7 to the RNA Column. Centrifuge at 10,000 × g for 1 minute at room temperature.
10. Repeat Step 9 until all of the sample has been transferred to the column. Discard the filtrate and reuse collection tube.
11. Add 600μl Buffer RWC to the column, Centrifuge at 10,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
12. Add 500μl Buffer RW2 to the column, Centrifuge at 10,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
13. Add 500μl Buffer RW2 to the column, Centrifuge at 10,000 × g for 1 minute at room

temperature. Discard the filtrate and reuse collection tube.

14. Centrifuge the empty Column at 10,000 × g for 2 minute at room temperature to dry the column matrix.

15. **Transfer the Column to a clean 1.5ml microcentrifuge tube. Add 30~50μl RNase Free Water directly to the center of the column membrane.** Let sit at room temperature for 2 minutes. Centrifuge at 10,000 × g for 1 minute at room temperature.

16. Store RNA 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 qiagen 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.

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..