HiPure Universal 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. A lternatively, an miRNA-enriched fraction and a total RNA (>200 nt) fraction can be purified separately. This Kit combines phenol/guanidine-based lysis of samples and silica membrane–based purification of total RNA. MagZol Reagent, included in the kits, is a monophasic solution of phenol and guanidine thiocyanate, designed to facilitate lysis of tissues, to inhibit RNases, and also to remove most of the cellular DNA and proteins from the lysate by organic extraction.

Kit Contents

Product	R431002	R431003	Contents
Preparation Times	50	250	—
HiPure RNA Mini Columns	100	2 x 250	Silica Column
2ml Collection Tubes	100	2 x 250	PP Column
MagZol Reagent	60 ml	270 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	30 ml	DEPC-Treated Water

Storage and Stability

MagZol Reagent should be stored at 2 – 8° C upon arrival. However, short-term storage (up to 24 weeks) at room temperature (15 – 25° C) does not affect its 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
- Microcentrifuge capable of at least 12,000 × g
- Chloroform

Protocol

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

   a) Tissue Samples

   Homogenize tissue samples in 1 mL of MagZol Reagent per 30~100mg of tissue using an appropriate mechanical homogenizer. Alternatively one can pulverize tissue in liquid nitrogen with mortar and pestle and transfer the powder to a clean 1.5 ml microcentrifuge tube. The sample volume should not exceed 10% of the volume of MagZol Reagent used.

   b) Cells Grown in Suspension

   Pellet cells by centrifugation. Lyse cells in MagZol Reagent by repetitive pipetting. Use 1 mL of the reagent per 5 x 10^6 of animal, plant or yeast cells, or per 1 x 10^8 bacterial cells. For plant, fungal, and yeast cells mechanical or enzymatic homogenization may be required.

   c) Cells Grown in Monolayer

   Lyse cells directly in a culture dish by adding 1 mL of MagZol Reagen to dish, and passing the cell lysate several times through a blue pipette tip. The amount of MagZol Reagent added is based on the area of the culture dish (~1 mL per 10 cm^2).

2. **Add 0.2 mL of chloroform per 1 mL of MagZol Reagent.** Cap sample tubes securely and shaking vigorously for 15 seconds by hand. Incubate at room temperature for 3 minutes.

3. **Centrifuge the samples at 12,000 x g for 15 minutes 4°C.** The mixture separates into a lower phenol-chloroform phase, an interphase, and an upper aqueous phase. RNA remains entirely in the aqueous phase.

- **Total RNA Isolation (including miRNA)**

4. **Transfer 500µl of the upper, aqueous phase to a new tube (not supplied). Add 1.5 volume of absolute ethanol** and mix thoroughly by vortexing. Do not centrifuge.

Precipitates may be visible after addition of ethanol. Resuspend precipitates completely by vigorous shaking and proceed immediately to step 5.

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

6. **Add 700µl of the sample from Step 4 to the Column.** Centrifuge at 10,000 × 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. Discard the filtrate and reuse collection tube. Proceed step 9.

- miRNA-Enriched Fractions Separate from Larger RNAs

4. **Transfer 500µl of the upper, aqueous phase to a new tube (not supplied). Add 150µl of absolute ethanol to the supernatant,** and mix thoroughly by vortexing. Do not centrifuge.

5. Insert a HiPure RNA Mini Column in a 2ml Collection Tube. **Add 650µl of the sample from Step 4 to the Column.** Centrifuge at 10,000 × g for 1 minute at room temperature.

   If purifying larger RNAs (>200 nt), save the first HiPure RNA Mini column for use in step 5 and Proceed step 9. (the spin column can be stored at 4°C or at room temperature, but not for long periods).

6. **Add 600µl of absolute ethanol to the flow-through, and mix well by pipetting.**

7. Insert another HiPure RNA Mini Column in a 2ml Collection Tube. **Add 700µl of the sample from Step 6 to the Column.** Centrifuge at 10,000 × 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. Discard the filtrate and reuse collection tube. Proceed step 9.

- Wash Column and Elute

9. **Add 650µl Buffer RWC to the column, Centrifuge at 10,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.

10. **Add 650µl Buffer RW2 to the column, Centrifuge at 10,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.

11. **Add 650µl Buffer RW2 to the column, Centrifuge at 10,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.

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

column matrix.

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

14. 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..