HiPure Liquid RNA (miRNA) Kit

Introduction

This Kit uses concentrated MagZol LS Reagent and silica spin column purification technology. It suites for high purity isolation of total RNA and miRNA from 0.25 ml liquid samples including fresh/frozen whole blood, plasma, buffy coat, cell suspensions and various body fluids. The entire extraction workflow can be completed within 40 minutes. Purified RNA is directly applicable to downstream experiments such as RT-PCR, Northern blot, poly-A+ mRNA purification, RNase protection assay and in vitro translation.

Kit Contents

Product	R416301	R416302	R416303
Preparation Times	20	50	250
HiPure RNA Mini Columns	20	50	250
2ml Collection Tubes	20	50	2 x 125
10 x RBC Lysis Buffer *	25 ml	50 ml	250 ml
MagZol LS Reagent	25 ml	50 ml	250 ml
Buffer RWC*	10 ml	20 ml	80 ml
Buffer RW2*	10 ml	20 ml	2 x 50 ml
RNase Free Water	5 ml	10 ml	30 ml

Storage and Stability

MagZol LS 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 their performance. The remaining kit components can be stored dry at room temperature (15–25°C) and are stable for at least 24 months under these conditions.

Materials and Equipment to be Supplied by User

- Dilute 10x RBC Lysis Buffer with ddH_2O to 1 x RBC Lysis Buffer and store at room temperature.

- Dilute Buffer RWC with 20ml (20 Preps), 40ml (50 Preps) or 160ml (250 Preps) absolute ethanol and store at room temperature
- Dilute Buffer RW2 with 40ml (20 Preps), 80ml (50 Preps) or 2 x 200ml (250 Preps) absolute ethanol and store at room temperature
- Microcentrifuge capable of at least 12,000 × g
- Chloroform
- Always maintain a ratio of 3:1 between the volume of MagZol LS Reagent and the sample
- Use cold MagZol LS Reagent if the starting material contains high levels of RNase
- MagZol LS Reagent is designed for processing liquid samples (blood and virus preparations, for example). Do not use MagZol LS Reagent undiluted with solid samples. Processing solid samples with MagZol LS Reagent results in decreased yield.

Isolation of Lymphocytes (require for 0.25-1ml whole blood)

1. **Transfer 0.25~1ml whole blood to a 15 ml centrifuge tube, add 5 volume of 1 x RBC Lysis Buffer and mix by vortexing.**

   10 x RBC Lysisi Buffer should be diluted to a concentration of 1 x before use.

   e.g. Add 5ml 1 x RBC lysis Buffer and 1ml whole blood into the 15ml centrifuge tube

2. **Incubate for 10–15 min on ice.** Mix by vortexing briefly 2 times during incubation
3. **Centrifuge at 500 x g for 10 min at 4°C. Remove the suppernatant by pietting and leave 250 µl liquid with the sediment.** Vortex for 5~10 sec to resespend the cells. completely remove and discard supernatant.

Protocol

1. **Transfer 750 µl MagZol LS Reagent into a 2.0 ml centrifuge tube.**
2. **Add suitable volume of liquid sample (following bellow table) to the tube and shake the tube up and down vigorously immediately for 5~10 sec to mix the sample. Vortex for 5~10 sec to completely disperse the pellet.**

sample	Sample volume	RNase Free Water	MagZol LS Reagent
Human whole blood	250 µl	-	750 µl
Cells resuspend mixture	250 µl	-	750 µl
Buffy Coat	250 µl	-	750 µl

Mammal blood	250 µl	-	750 µl
Avian blood	50 µl	200 µl	750 µl
Other non-mammalian organisms blood	100 µl	150 µl	750 µl
Other liquid sample	250 µl	-	750 µl
Serum, plasma	250 µl	-	750 µl
Saliva/body fluid	250 µl	-	750 µl

If the sample volume is less than 250µl, add the appropriate volume of RNase-free Water to get 250µl volume.

Samples can be stored at 2~8°C for one week or at – 20°C/-80°C for up to a year.

3. **Add 0.2 ml of chloroform to the sample.** Cap sample tubes securely and shaking vigorously for 15 seconds by hand. Incubate at room temperature for 3 minutes.

4. **Centrifuge the samples at 12,000 x g for 15 min at 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.

5. Transfer the upper, aqueous phase to a new centrifuge tube (not supplied). **Add 0.5 or1.5 volume of absolute ethanol and mix thoroughly by shaking up an down 6~8 times. Do not centrifuge.**

- If miRNA require in the result, add 1.5 volume of absolute ethanol to the tube.
- If only requires mRNA (>200nt), add 0.5 volume of absolute ethanol to tube. This would remove 5S RNA, tRNA, and other short fragment RNA (<200nt) efficiently and increase mRNA purity.
- Precipitates may be visible after addition of ethanol. Resuspend precipitates completely by vigorous shaking and proceed immediately to step 6.

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

7. **Add 700µl of the sample from Step 5 to the Column.** Centrifuge at 12,000 × g for 30~60 sec 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.

9. **Add 500µl Buffer RWC to the column, Centrifuge at 12,000 × g for 30 sec at room temperature.** Discard the filtrate and reuse collection tube.

10. **Add 500μl Buffer RW2 to the column, Centrifuge at 12,000 × g for 30 sec at room temperature.** Discard the filtrate and reuse collection tube.

11. Repeat Step 10 once.

12. Centrifuge the empty Column at 12,000 × g for 3 min at room temperature to dry the column matrix.

13. **Transfer the Column to a clean 1.5ml centrifuge tube. Add 30~50μl RNase Free Water directly to the center of the column membrane.** Stay at room temperature for 2 minutes. Centrifuge at 12,000 × g for 1 min at room temperature.

14. **Repeat step 13 using another volume of RNase-free water, or using the eluate from step 13** (if high RNA concentration is required).

15. Store RNA at -20℃ or -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 RWC：** In subsequent preparations, incubate the RNA Mini Column for 5 min at room temperature after addition of Buffer RWC and before centrifuging.