HiPure Liquid RNA(miRNA) Kit

Introduction

The HiPure Liquid RNA(miRNA) Kit integrates phenol/guanidine-based sample lysis and silica membrane purification of total RNA. MagZol LS Reagent, included in the kits, is a monophasic solution of phenol and guanidine thiocyanate, designed to facilitate lysis of Liquid sample and inhibit RNases. The high lysis efficiency of the reagent and the subsequent removal of contaminants by organic phase extraction enable the use of up to 0.25ml Liquid sample per Mini spin column.

Principle

0.25ml liquid samples are homogenized in 0.75ml MagZol LS Reagent. After addition of chloroform, the homogenate is separated into aqueous and organic phases by centrifugation. The upper, aqueous phase is extracted, and ethanol is added to provide appropriate binding conditions. The sample is then applied to the spin column, where the total RNA (up to 100 µg) binds to the membrane and phenol and other contaminants are efficiently washed away. High-quality RNA is then eluted in 30–100 µl of RNase-free water water

Kit Contents

Product	R416302	R416303
Preparation Times	50	250
HiPure RNA Mini Columns	50	250
2ml Collection Tubes	50	250
MagZol LS Reagent	60 ml	270 ml
Buffer RWC	20 ml	60 ml
Buffer RW2*	20 ml	2 x 50 ml
RNase Free Water	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 18 months under these conditions.

Materials and Equipment to be Supplied by User

- Dilute Buffer RWC with 40ml (50 Preps) or 120ml (250 Preps) absolute ethanol and store at room temperature
- Dilute Buffer RW2 with 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.

Protocol

1. Pipet 750 µl MagZol LS Reagent into a 2.0 ml microcentrifuge tube.

2. Add 250 µl liquid sample to the microcentrifuge tube. Homogenize the sample by pipetting up and down several times.

   Use up to 250 µl plasma, serum, or body fluids, or up to 5 x 10^6 lymphocytes or Culture Cells in 250 µl Water or saline Solution. If the sample volume is less than 250µl, add the appropriate volume of Water. Biological fluids with high levels of contamination material (whole blood, bone marrow for instance) should be diluted 1:1 with RNase-free water.

   Samples can be stored at 4°C overnight or at – 20°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 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.

5. Transfer 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 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 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.

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 80% ethanol 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. **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℃.

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.

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..