HiPure Universal RNA Kit

Introduction

The Kit integrates phenol/guanidine-based lysis and silica membrane purification of total RNA. MagZol Reagent, included in the kits, is a monophasic solution of phenol and guanidine thiocyanate, designed to facilitate lysis of samples and inhibit RNases. The high lysis efficiency of the reagent and the subsequent removal of contaminants by organic phase extraction enable extracting up to 200ug total purified RNA from less than 100mg animal, plant, fungal samples, bacteria, cells and other samples.

Principle

Tissue samples (10–100mg) are homogenized in MagZol 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	R413002	R413003	Contents
Preparation Times	50	250	—
HiPure RNA Mini Columns	50	250	Silica Column
2ml Collection Tubes	50	250	PP Column
MagZol Reagent	54 ml	270 ml	Guanidine Salt/Phenol
Buffer RW1	40 ml	200 ml	Guanidine Salt
Buffer RW2*	10 ml	50 ml	Tris/EDTA
RNase Free Water	6 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 12 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

- Add 80ml/200ml absolute ethanol to the bottle of Buffer RW2 and store at room temperature.See RW2 label.
- Microcentrifuge capable of at least 12,000 × g
- Chloroform
- Absoluet ethanol

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. If ceramic mortar and pestle are not available, homogenize the sample in the microfuge tube using a disposable microtube pestle. The sample volume should not exceed 10% of the volume of MagZol Reagent used.

   Optional: For samples containing a relatively high content of fat, proteins, polysaccharides, or extracellular material, centrifuge the homogenate at 12,000 x g for 10 min at 4°C to remove insoluble material. Carefully transfer the supernatant to a new tube, and proceed to step 2.

   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. Washing cells before addition of MagZol Reagent should be avoided as this increases the possibility of mRNA degradation and RNase contamination. 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 Reagent to a 3.5 cm diameter 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). An insufficient amount of MagZol Reagent may result in contamination of the isolated RNA with DNA. Always use more MagZol Reagent if in the lysate is too viscous to aspirate with a pipette.

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. *This step is critical - do not change it.*

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.

4. **Transfer the upper, aqueous phase to a new tube (not supplied). Add 1 volume (usually 550 µl) of Buffer RW2,** 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 600µl 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.

8. **(Optional) Add 650µl Buffer RW1 to the column, Centrifuge at 10,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.

9. **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.

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

11. **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.

12. Repeat step 11 using another volume of RNase-free water, or using the eluate from step 11 (if high RNA concentration is required).

13. 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 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..