HiPure RNA Clean Up Kit

Introduction

HiPure RNA Clean Up Kit provides a rapid and easy method for the purification and concentrate RNA from enzymatic reactions or for desalting the RNA samples. RNA purified using HiPure RNA Clean Up Kit is ready for all downstream applications such as RT-PCR, Northern blotting, mRNA purification,nuclease protection,and in vitro translation.

Principle

The HiPure system uses a simple bind-wash-elute procedure. Binding buffer is added directly to the sample or other enzymatic reaction, and the mixture is applied to the column.Nucleic acids adsorb to the silica membrane in the high-salt conditions provided by the buffer. Impurities are washed away and pure RNA is eluted with a small volume of low-salt buffer provided or water, ready to use in all subsequent applications.

Kit Contents

Product	R214401	R214402	R214403
Purification times	20 Preps	50 Preps	250 Preps
Buffer GXP	10 ml	30 ml	120 ml
Buffer RW2*	10 ml	20 ml	2 x 50 ml
RNase-Free Water	10 ml	20 ml	60 ml
HiPure RNA Mini Columns I	20	50	250
2 ml Collection Tubes	20	50	250

Storage and Stability

The Kit can be stored dry at room temperature (15–25°C) and are stable for at least 18 months under these conditions.The entire kit can be stored at 2–8°C, but in this case buffers should be redissolved before use. Make sure that all buffers are at room temperature when used.

Materials and Equipment to be Supplied by User

- Dilute Buffer RW2 with 40ml (20 Preps) or 80ml (50 Preps) or 2 x 200ml (250 Preps) 100% ethanol and store at room temperature。
- Microcentrifuge capable of at least 13,000 × g
- Absolute ethanol

Binding Capacity

HiPure RNA Mini column I can bind ~50ug DNA.

Protocol

1. Briefly centrifuge the reactions or RNA Products to collect any drops from the inside of the lid. Determine the volume of your sample Volume,and transfer the sample into a clean 1.5ml microcentrifuge tube.
2. **Add RNase Free Water to a total volume of 100ul.**
3. **Add 300μl Buffer GXP to the sample, Vortex to mix thoroughly. Incubate for 3 min.**
4. **Add 250μl absolute ethanol (for miRNA Clean up, add 600ul 100% ethanol) to the sample and vortex to mix well.** Briefly centrifuge the tube to collect any drops from the inside of the lid.

Spin protocol

5. Insert a HiPure RNA Column I in a 2ml Collection Tube.
6. **Add ≤ 700μl solution from Step 4 to the Column.** Centrifuge at 8,000 × 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.
8. **Add 650μl Buffer RW2(Diluted with 100% ethanol prior to use) to the column.** Centrifuge at 8,000 × g for 1 minute at room temperature.Discard the filtrate and reuse collection tube.

9. **Add 650μl Buffer RW2(Diluted with 100% ethanol prior to use) to the column.** Centrifuge at 8,000 × g for 1 minute at room temperature.Discard the filtrate and reuse collection tube.

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

11. **Transfer the HiPure RNA Mini Column I to a clean 1.5ml microcentrifuge tube.Add 15-30μl RNase Free Water directly to the center of the column membrane.**Let sit at room temperature for 2 minutes.

12. Centrifuge at 12,000 × g for 1 minute at room temperature. Store RNA at -20℃.

Vaccum protocol

5. Prepare the vacuum manifold following manual.

6. Insert up to HiPure RNA columns into the luer extensions of the Vacuum manifolds. Close unused positions with luer caps, and then connect manifolds to a vacuum source.

7. **load the samples from step 4 into the columns by decanting or pipetting,and then apply vacuum.** After the samples have passed through the column, switch off the vacuum source. The maximum loading volume of the column is 800 μl. For sample volumes greater than 800 μl, simply load again.

8. To wash, add 0.75ml of Buffer RW2 to each column and apply vacuum.

9. To wash, add 0.5ml of Absolute ethanol to each column and apply vacuum.

10. Transfer each column to a 2ml collection tubes. Centrifuge for 1 min at 17,900 x g (13,000 rpm).

11. Place each column into a clean 1.5 ml microcentrifuge tube.

12. To elute RNA, add 10~50μl RNase Free water to the center of the membrane. let the column stand for 1 min, and then centrifuge.

Troubleshooting Guide

1. Low or no recovery

- **Buffer RW2 did not contain ethanol:** Ethanol must be added to Buffer DW2 before used. Repeat precedweure with correctly prepare Buffer PE.
- **Inappropriate Elution Buffer:** RNA will only be eluted efficiently in the presende of low salt buffer or Water.
- **Sample volume too high or low:** for reaction cleanup, The sample volume must be in the range of 20~200ul.

2. RNA does not perform well (e.g. in ligation reaction)

- **Salt concentration in eluate too high**: Modify the wash step by incubating the column for 5 min at room temperature after adding 650ul 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.
- **Eluate contaminated with Primer-dimers:** Primer-dimers are >20bp, and are not completely removed. After the binding step, wash the Column with 700ul of a 35% guanidine hydrochloride solution. Continue with the Buffer RW2 wash step.