HiPure Viral RNA Kit

Introduction

Hipure viral RNA kit is suitable for purifying viral RNA from samples such as acellular body fluid or culture medium. The kit is based on silica gel column purification technology. It requires no toxic phenol chloroform extraction and time-consuming alcohol precipitation in the extraction. The whole extraction process takes only 25 minutes. The kit is suitable for extracting viral RNA from 1-140μl serum, plasma, urine, acellular fluid or culture medium. The product has successfully extracted hepatitis B A/C, hepatitis C RNA, SARS and HIV. The obtained RNA can be directly used for RT-PCR, Northern hybridization and virus detection.

Kit Contents

Product	R417102	R417103
Purification times	50 Preps	250 Preps
HiPure Viral Micro Columns	50	250
2ml Collection Tubes	100	500
Buffer VRL	50 ml	200 ml
Carrier RNA	310 μg	3 x 310 μg
Buffer VHB*	13 ml	110 ml
Buffer RW2*	20 ml	2 x 50 ml
Nuclease Free Water	10 ml	30 ml
Protocol	1	1

Storage and stability

Carrier RNA 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 their 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.

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Materials and Equipment to be Supplied by User

- RNase Free 1.5ml Centrifuge tube
- 96~100% ethanol
- RNase Free tips
- Centrifuge device (<15,000 x g)
- Heat block or water bath capable of 55℃ and 90℃
- Dissolve Carrier RNA with Nuclease Free Water by vortex before use, final concentration is 1μg/μl. Store at -20°C. Do not multi freeze thawing.
- Add 17ml (50 preps) or 140ml (250 preps) 100% ethanol to the bottle of Buffer VHB and store at room temperature.
- Add 80ml (50 preps) or 2 x 200ml (250 preps) 100% ethanol to the bottle of Buffer RW2 and store at room temperature.

Protocol

- Prepare Buffer VRL/Carrier RNA mixture: Carrier RNA can increase yield of Viral RNA in extraction. Add 1ml Buffer VRL to 4μl Carrier RNA(1μg/μl). Buffer VRL/Carrier RNA mixture can stable at 2-8℃ for 1 month. There may be sediment in the mixture when store at low temperature. Incubate at 60℃ for 3 minutes to dissolve sediment completely before use.

1. Add 560μl Buffer VRL (include Carrier RNA) to a 1.5ml centrifuge tube.

   Note: if sample is over 140μl, increase Buffer VRL/Carrier RNA amount accordingly. IF sample is less than 140μl, add Buffer PBS to get a final 140μl volume.

2. Add 140μl sample such as plasma, serum, urine, acellular culture supernatant or other body fluids to the tube. Vortex for 20 seconds..

   Note: To lyse the cells completely, vortex the tube immediately after sample added. Frozen samples can be only thawed 1-2 times.

3. Stay at room temperature (15-25°C) for 10 minutes.

   Note: Viral samples will be lysed completely after stay at room temperature for 10 minutes. Samples can be store at -20℃ for long time storage at this step. If the sample still not clear after setting, centrifuge at 14,000 x g for 5 minutes to remove undissolved materials to avoid clogging on columns.

4. Add 560μl 100% ethanol to mixture, vortex for 20 seconds.

5. Insert a HiPure Viral Micro Column in a 2ml Collection Tube. Transfer 700μl mixture to the column , centrifuge at 10,000 x g for 30~60 seconds. Discard the filtrate and reuse collectio tube.

6. Transfer the rest mixture to the column, centrifuge at 10,000 x g for 30-60 seconds. Discard the filtrate and insert the column to a new 2ml Collection Tube.

7. Add 500μl Buffer VHB (Diluted with 100% ethanol before use) to the column. Centrifuge at 10,000 x g for 30~60 senconds. Discard the filtrate and reuse collection tube.

8. Add 500μl Buffer RW2 (Diluted with 100% ethanol before use) in the column. Centrifuge

at 8,000 x g for 30~60 senconds. Discard the filtrate and reuse collection tube.

9. Repeat step 8.
10. Centrifuge the empty Column at 13,000 x g for 3 minutes to dry the column matrix.
11. **Transfer the Column to a clean 1.5ml centrifuge tube. Add 15~30μl RNase Free Water directly to the center of the column membrane.** Stay at room temperature for 2 minutes.
12. Centrifuge at 13,000 x g for 1 minute at room temperature. Discard the column and store RNA at -20℃.

Troubleshooting Guide

1. Clogged HiPure Viral Micro Column

- **Too much samples:** for anticoagulant blood, sample amount should be at 100~150μl. Use non-cell samples such as plasma, serum, supernatant of tissue homogenate.
- **Particles in samples**: after step 3, centrifuge at 14,000 x g for 5 minutes, transfer the supernatant to a new centrifuge, and follow step 4.
- **Poor lysis for sample:** samples and Buffer VRL are not mixed thoroughly. Take new sample, after adding Buffer VRL, mix up side down for 3~5 times, and vortex at maximum speed lyses sample completely.

2. Poor performance in downstream applications

- **Samples frozen and thawed more than once:** repeated freezing and thawing should be avoid. Used fresh samples or samples thawed only once.
- **Over use of Carrier RNA:** adjust Carrier RNA amount according to RT-PCR sensitivity.
- Buffer VHB/RW2 did not add with 100% ethanol correctly before use.
- **Low concentraction of RNA:** reduce RNase Free Water volume for elution to increase RNA conc.