HiPure Blood RNA Midi Kit B

Introduction

The Kit provides fast purification of high-quality RNA from whole blood and other liquid samples. This kit integrates two high efficiency RNA extraction technology, combing one-step RNA extraction with silica-membrane spin columns purification. RNA purified using the HiPure RNA System is ready for applications such as RT-PCR, Northern blotting, poly A+ RNA (mRNA) purification, nuclease protection, and in vitro translation.

Kit Contents

Product	R416401B	R416402B	R416403B
Preparation Times	10	50	250
HiPure RNA Midi Columns I	10	50	5 x 50
15ml Collection Tubes	20	2 x 50	10 x 100
MagZol 3BD	85 ml	420 ml	4 x 550 ml
Buffer BCP2	15 ml	40 ml	210 ml
DNase I	600 µl	3 x 600 µl	15 x 600 µl
DNase buffer	5 ml	20 ml	100 ml
Buffer RW1	50ml	250 ml	2 x 550 ml
Buffer RW2*	10 ml	50 ml	5 x 50 ml
RNase Free Water	10 ml	20 ml	60 ml

Storage and Stability

Buffer BCP2 and MagZol 3BD should be stored at 2–8°C upon arrival. DNase I should be stored at -20°C. However, short-term storage (DNase I up to 1 weeks, Buffer BCP2 and MagZol 3BD up to 8 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 24 months under these conditions.

Materials and Equipment to be Supplied by User

- Add 40ml(10 Preps), 200ml (50 Preps) or 5 x 200ml (250 Preps) 100% ethanol to the bottle of Buffer RW2 and store at room temperature
- Microcentrifuge capable of at least 12,000 × g
- Isopropanol

Protocol

This protocol is suitable for high purity RNA extraction from 5.0ml fresh whole blood sample. It is also suitable for other mammalian blood samples

1. **Add 7 ml MagZol BD3 into a 15 ml centrifuge tube, then add 5 ml anticoagulated blood, buffy coat, serum, plasma, or other liquid samples.** Vigorously shake up and down for 15 seconds immediately, followed by vortexing for 15 seconds, and incubate in a 60°C water bath for 10 minutes.

- Collect whole blood in EDTA anticoagulant vacuum blood collection tubes and transfer it to centrifuge tubes containing MagZol 3BD as soon as possible. Thorough mixing is critical for RNA yield.

- **Low-temperature blood storage method 1:** Transfer 3 ml blood to a 15 ml centrifuge tube and store at -70 °C. For RNA extraction, add 3.6 ml MagZol 3BD to the frozen sample without thawing, then invert or shake repeatedly until completely thawed. Do not thaw blood samples without lysis reagent, as this will cause RNA degradation.

- **Low-temperature blood storage method 2:** Transfer 3 ml blood to a 15 ml centrifuge tube, add 3.6 ml MagZol 3BD, shake vigorously up and down 10–15 times, vortex for 15 seconds, and incubate in a 60 °C water bath for 10 minutes. The obtained lysate can be stored stably for at least 2 years at -70 °C, 6 months at -20 °C, 10 days at 2–8 °C, and more than 5 days at room temperature.

2. **Add 700 ul Buffer BCP2 to the sample** and shake vigorously up and down for 15 seconds. Centrifuge at 4,000–5,000 × g at room temperature for 10 minutes.

3. **Transfer 5 ml supernatant to a new 15 ml centrifuge tube, add 1.8 ml isopropanol.** Invert the tube 3–5 times to mix thoroughly.

4. Place a HiPure RNA Midi Column I into a 15 ml collection tube (supplied). Transfer half of the mixture into the column, and centrifuge at 4,000 × g for 2 minutes.

5. Discard the flow-through, reassemble the column into the collection tube, transfer the remaining mixture onto the column, and centrifuge at 2,000 × g for 2 minutes.

6. Discard the flow-through and place the column back into the collection tube. **Add 1 ml Buffer RW1 to the column,** then centrifuge at 2,000 × g for 10 minutes.

7. Prepare the DNase Mixture: Combine 300 µl DNase Buffer with 30 µl DNase I, and pipette up and down 3 times to mix well. Add the mixture directly to the center of the column membrane, and incubate at room temperature for 15 minutes.

8. **Add 3 ml Buffer RW1 to the column** and centrifuge at 2,000 × g for 2 minutes.

9. Discard the flow-through and reassemble the column onto the collection tube. **Add 4 ml Buffer RW2 (diluted with ethanol) to the column,** and centrifuge at 4,000 × g for 10 minutes.

10. Transfer the column to a new 15 ml centrifuge tube. **Add 100 µl RNase Free Water to the center of the column membrane** and stay at room temperature for 2 minutes. Centrifuge at 4,000 × g for 3 minutes.

    When centrifuge speed at 4,000~5,000 x g, will get ~50 µl product from 100 µl elution buffer. When centrifuge speed at 8,000 x g, will get ~ 80 µl product from 100 µl elution buffer..

11. Add an additional 50 µl RNase Free Water to the center of the column membrane, incubate at room temperature for 2 minutes, and centrifuge at 4,000 × g for 3 minutes. Discard the column and store the purified RNA at −80 or −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..