HiPure Blood RNA Mini Kit

Introduction

The Kit provides fast purification of high-quality RNA from whole blood and cells using silica-membrane spin columns with a binding capacity of 100ug RNA. There is no need for phenol/chloroform extractions and time-consuming steps such as CsCl gradient ultracentrifugation, or precipitation with isopropanol or LiCl are eliminated. 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.

Principle

The Kit simplifies isolation of RNA from blood with a fast spin-column procedure. Red blood cells are selectively lysed and white cells collected by centrifugation. White cells are then lysed using highly denaturing conditions which immediately inactivate RNases. After homogenization using the DNA spin column, the sample is applied to the RNA column. Total RNA binds to the membrane and contaminants are washed away, leaving pure RNA to be eluted in 30–100 µl RNase-free water (provided with the kit) for direct use in any downstream application.

Kit Contents

Product	R416100	R416102	R416103	Contents
Preparation Times	20	50	250	-
HiPure DNA Mini Columns	20	50	250	Silica Column
HiPure RNA Mini Columns I	20	50	250	Silica Column
2ml Collection Tubes	40	100	500	PP Column
10 x Buffer RBC*	20 ml	50 ml	3 x 100 ml	NH_4AC
RTL Lysis Buffer	15 ml	50 ml	250 ml	Guanidine Salt
Buffer RW1	15 ml	50 ml	200 ml	Guanidine Salt
Buffer RW2*	6 ml	20 ml	2 x 50 ml	Tris/EDTA
RNase Free Water	5 ml	10 ml	30 ml	Water

Storage and Stability

HiPure Blood RNA Mini Kit components can be stored dry at room temperature (15–25°C) and are stable for at least 18 months under these conditions. Make sure that all buffers are at room temperature when used. During shipment, crystals or precipitation may form in the RTL Lysis Buffer. Dissolve by warming buffer to 37°C.

Materials and Equipment to be Supplied by User

- Add 24ml(20 Preps), 80ml (50 Preps) or 2 x 200ml (250 Preps) 100% ethanol to the bottle of Buffer RW2 and store at room temperature
- Dilute 10 x Buffer RBC with ddH_2O to 1 x Buffer RBC and store at room temperature.
- Microcentrifuge capable of at least 12,000 × g
- (Optional) 2-mercaptoethanolor 1M DTT can be added to an aliquot of RTL Lysis Buffer before use. Add 20µl 2-mercaptoethanol or 1M DTT per 1mL RTL Lysis Buffer. This mixture can be stored for 2 weeks at room temperature

Protocol

1. **Transfer 1-1.5 ml whole blood to 15 ml centrifuge tube. Add 5 volumes of 1x Buffer RBC and mix votexing.**
   Up to 1.5 ml of healthy blood (typically 4000–7000 leukocytes per microliter) can be processed. Reduce amount appropriately if blood with elevated numbers of leukocytes is used. Dilute 10 x Buffer RBC to 1 x Buffer RBC with ddH_2O before use.
2. **Incubate for 10–15 min on ice.** Mix by vortexing briefly 2 times during incubation.
3. **Centrifuge at 400 x g for 10 min at 4°C, and completely remove and discard supernatant.**
   Leukocytes will form a pellet after centrifugation. Ensure supernatant is completely removed. Trace amounts of erythrocytes, which give the pellet a red tint, will be eliminated in the following wash step.
4. **Add 2 volume of 1 x Buffer RBC to the cell pellet (use 2 volumes of 1 x Buffer RBC per volume of whole blood used in step 1).** Resuspend cells by vortexing briefly.
5. **Centrifuge at 400 x g for 10 min at 4°C, and completely remove and discard supernatant.**
   Incomplete removal of the supernatant will interfere with lysis and subsequent binding of RNA to the column, resulting in lower yield.

6. **loosen the pelleted leukocytes thoroughly by flicking the tube and add 0.7ml RTL Lysis Buffer .** Vortex or pipet to mix.
   Homogenized cell lysates can be stored at –70°C for several months. Frozen lysates should be incubated at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation, which may compromise RNA integrity.

7. **Homogenize the lysate by pass the lysate at least 5 times through a blunt 20-gauge needle fitted to an RNAse-free syringe.**

8. Insert a HiPure DNA Mini Column in a 2ml Collection Tube.

9. Transfer the lysate to DNA Mini column placed in a 2 ml collection tube (supplied). Centrifuge for 60 s at ⩾12,000 x g . Discard the column, and save the flow-through.

10. **Add eqaul volume of 70% ethanol to the flow-through.** mix immediately by pipetting.

11. Insert a HiPure RNA Mini Column I in a 2ml Collection Tube.

12. **Add up to 700µl of the sample from Step 10 to the Column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.

13. Repeat Step 12 until all of the sample has been transferred to the column.

14. **Add 650µl Buffer RW1 to the column, centrifuge at 12,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.

15. **Add 650µl Buffer RW2 to the column, Centrifuge at 12,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.

16. **Add 650µl Buffer RW2 to the column, Centrifuge at 12,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.

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

18. **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.

19. Centrifuge at 12,000 × g for 1 minute at room temperature. 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..