HiPure Paxgene Blood RNA Kit

Introduction

The Kit provides fast purification of high-quality RNA from paxigene Blood RNA Tube using silica-membrane spin columns. RNA purified using the HiPure 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 is for the purification of total RNA from 2.5 ml human whole blood collected in a PAXgene Blood RNA Tube. Purification begins with a centrifugation step to pellet nucleic acids in the PAXgene Blood RNA Tube. The pellet is washed and resuspended, and incubated in optimized buffers together with proteinase K. DNA wash The lysate is passed through a DNA Mini column. Ethanol is added to adjust binding conditions, and the lysate is applied to a column. RNA is selectively bound to the silica membrane as contaminants pass through. Remaining contaminants are removed in several efficient wash steps. Between the first and second wash steps, the membrane is treated with DNase I to remove trace amounts of bound DNA. After the wash steps, RNA is eluted in elution buffer and heat-denatured.

Kit Contents

Product	R416802	R416803
Preparation Times	10	50
HiPure RNA Mini Columns I	10	50
gDNA Filter Mini Columns	10	50
2ml Collection Tubes	30	150
RNase Free Water	60 ml	250 ml
Buffer MBR1 (RTL Lysis Buffer)	10 ml	30 ml
Buffer MBR2 (RNA Digestion Buffer)	5 ml	15 ml
Buffer RW1	15 ml	60 ml
Buffer RW2*	6 ml	20 ml
Proteinase K	12 mg	50 mg
Protease Dissolve Buffer	1.8 ml	5 ml
DNase I	120 µl	600 µl
DNase Buffer	6 ml	30 ml

Storage and Stability

Proteinase K 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, Proteinase K 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 18 months under these conditions.

Materials and Equipment to be Supplied by User

- Dilute Buffer RW2 with 24ml (10 Preps) or 80ml (50 Preps) 100% ethanol and store at room temperature.
- Microcentrifuge capable of at least 12,000 × g
- Ensure that the PAXgene Blood RNA Tubes are incubated for at least 2 hours at room temperature after blood collection to ensure complete lysis of blood cells. Incubation of the PAXgene Blood RNA Tube overnight may increase yields. If the PAXgene Blood RNA Tube was stored at 2–8°C, –20°C or –70°C after blood collection, first equilibrate it to room temperature and then store it at room temperature for 2 hours before starting the procedure.

Protocol

1. **Centrifuge the PAXgene Blood RNA Tube for 10 minutes at 3000–5000 x g using a swing-out rotor**. Remove the supernatant by decanting or pipetting.

   Ensure that the blood sample has been incubated in the PAXgene Blood RNA Tube for a minimum of 2 hours at room temperature (15–25°C) in order to achieve complete lysis of blood cells.

2. **Add 4 ml RNase-Free Water to the pellet and close the tube using a fresh secondary BD Hemogard closure (no supplied with the kit).** If the supernatant is decanted, take care not to disturb the pellet and dry the rim of the tube with a clean paper towel.

3. **Vortex until the pellet is visibly dissolved and centrifuge for 10 minutes at 3000–5000 x g using a swing-out rotor.** Remove and discard the entire supernatant.

   Small debris remaining in the supernatant after vortexing but before centrifugation will not affect the procedure. Incomplete removal of the supernatant will inhibit lysis and dilute the lysate, and therefore affect the conditions for binding RNA to the PAXgene membrane.

4. **Add 350 µl Buffer MBR1 and vortex until the pellet is visibly dissolved.** Pipet the sample into a 1.5 ml microcentrifuge tube.

5. **Add 300 µl Buffer MBR2 and 40 µl proteinase K. Mix by vortexing for 5 seconds and incubate for 10 minutes at 55°C using a shaker–incubator at 400–1400 rpm.**

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

7. **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.

8. **Add 350 µl absolute ethanol to the flow-through.** mix immediately by pipetting.

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

10. **Add up to 700µl of the sample from step 8 to the Column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.

11. Repeat Step 10 until all of the sample has been transferred to the column.

12. **Add 350µl Buffer RW1 to the column and centrifuge at 12,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.

13. **Add 10µl DNase I to 90 µl DNase Buffer in a 1.5 ml microcentrifuge tube.** Mix by gently flicking the tube and centrifuge briefly to collect residual liquid from the sides of the tube.

14. **Pipet DNase I mix (100 µl) directly onto the RNA spin column membrane and place on the benchtop (20–30°C) for 15 minutes**.

15. **Pipet 500 µl Buffer RW1 into the RNA spin column and incubate for 1 min. Centrifuge for 1 minute at 12,000 x g.** Place the spin column in a new 2 ml processing tube and discard the old processing tube containing flow-through.

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. **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.

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

19. **Transfer the column to a clean 1.5ml microcentrifuge tube. Add 40 µl RNase Free Water directly onto the spin column membrane.** Centrifuge for 1 minute at 12,000 x g to elute the RNA.

20. Repeat the elution step using 40µl RNase Free Water and the same microcentrifuge tube.

21. **Incubate the eluate for 5 minutes at 65°C in the shaker–incubator without shaking.** After incubation, chill immediately on ice.

This incubation at 65°C denatures the RNA for downstream applications. Do not exceed the incubation time or temperature.

22. If the RNA samples will not be used immediately, store at –20°C or –70°C.

    Since the RNA remains denatured after repeated freezing and thawing, it is not necessary to repeat the incubation at 65°C. For accurate quantification of RNA by absorbance at 260 nm, we recommend diluting samples with 10 mM Tris-HCl, pH 7.5*. Diluting the sample in RNase-Free Water may lead to inaccurately low values.

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..