AipPure PAX Blood RNA Extraction Kit (For PAXgene Blood DNA Tube)

Operating Instruction

◆**Catalog No：RE233**

◆**Kit Contents and Storage：**

Kit Contents	Storage	50 Preps (RE233-01)
Resuspension Buffer	RT	20mL
Binding Buffer	RT	50mL
Buffer RW1	RT	40mL
Wash Buffer RW	RT	10mL 42mL absolute ethanol was adde before first use
RNase-free Water	RT	10mL
DNase Buffer	–20℃	1.25mL x 2
RNase free DNase I	–20℃	250μL
Proteinase K(20mg/mL)	RT/–4℃	1mL
RNA Binding Columns	RT	50

Note: Proteinase K is stored in glycerol storage buffer. Store at RT for 6 months, at 4 °C for 1 year, –20 °C for 2 years.

All reagents, when store in indicated temperature, are stable for 12 months.

◆**Description：**

PAX Blood RNA Kit is designed for isolation of total RNA from blood samples stored in preservation reagents and PAXgene® Blood RNA Tubes. This procedure completely removes contaminants and enzyme inhibitors producing high-quality RNA. RNA purified using the PAX Blood RNA Kit is ready for applications such as RT-PCR.

The samples are removed from the preservation reagents. For blood samples stored in PAXgene® Blood RNA Tubes, the cells are collected by centrifugation. Samples are washed and lysed under an optimized buffer containing Proteinase K. The samples are centrifuged to remove cell debris and other particulates. After adjusting the binding conditions with ethanol, the samples are loaded on the RNA binding column. With a brief centrifugation or vacuum step, the samples pass through the column matrix which binds the RNA. Genomic DNA is removed with an on-the-column DNase I digestion treatment. After three wash steps,

purified RNA is eluted with RNase-free water.

◆Materials and Equipment to be Supplied by User：

1. Microcentrifuge capable of 13,000xg
2. 100% ethanol
3. RNase-free filter pipette tips
4. RNase-free water
5. 1.5 or 2.0mL microcentrifuge tubes
6. Shaking incubators or heat blocks capable of 55°C, 65°C
7. Centrifuge with swing-bucket rotor capable of 5,500xg

◆Procedure：

Note：

⇨ **Before the first use, add the indicated amount of ethanol into Wash Buffer RW bottles, mix well, and mark the bottle with a check.**

⇨ **Heat the incubators or heat blocks to 55°C, 65°C.**

1. Centrifuge the PAXgene® Blood RNA Tube for 10 minutes at 3,000-5,000xg using a swing-out rotor.
2. Aspirate and discard the supernatant.
3. Add 4mL RNase-free water. Vortex to completely resuspended the pellet.
4. Centrifuge the PAXgene® Blood RNA Tube for 10 minutes at 3,000-5,000xg using a swing-out rotor.
5. Aspirate and discard the supernatant.
   Note: Incomplete removal of the supernatant will reduce the lysis efficiency and dilute the lysate, thereby reducing the RNA yield.
6. Add 350µL Resuspension buffer. Vortex to completely resuspend the pellet.
7. Transfer the sample into a new 1.5mL microcentrifuge tube.
8. Add 300µL Binding Buffer and 20µL Proteinase K Solution. Vortex for 5 seconds to mix thoroughly.
9. Incubate at 55°C for 10 minutes using a shaking incubator.
10. **Optional:** Pass the lysate at least 5 times through a 20-gauge needle (0.9 mm diameter) fitted to an syringe or homogenize with an electronic tissue homogenizer. This step shears genomic DNA, reduces the viscosity of the lysates, and increases the yields.
11. Centrifuge the homogenized lysate at 13,000 rpm for 3 min. Transfer the supernatant into a new centrifuge tube.

12. Add 0.5 volumes 100% ethanol. Vortex to mix thoroughly.
13. Transfer up to 700µL mixture into a RNA binding column placed in a 2mL collection tube (provided).
14. Centrifuge at maximum speed for 1 minute.
15. Aspirate and discard the filtrate and reuse the collection tube.
16. Repeat Steps 13-15 until the remaining sample has been transferred to RNA binding column.
17. Add 350µL Buffer RW1.
18. Centrifuge at maximum speed for 1 minute.
19. Aspirate and discard the filtrate and reuse the collection tube.
20. For each of the RNA binding column, prepare the DNase I digestion reaction mix as follows:

Buffer	Volume per Prep	10 Preps
DNase I Buffer	45µL	450µL
RNase-free DNase I	5µL	50µL
Total volume	50µL	500µL

Important Notes:

- DNase I is very sensitive and prone to physical denaturing. Do not vortex the DNase I mixture. Mixgently by inverting the tube.
- Freshly prepare DNase I stock solution right before RNA isolation.
- Standard DNase buffers are not compatible with on-membrane DNase I digestion. The use of other buffers may affect the binding of RNA to the matrix and may reduce RNA yields and purity.
- All steps must be carried out at room temperature. Work quickly, but carefully.

21. Pipet 50µL DNase I digestion reaction mix directly onto the centre surface of the RNA binding column.

    Note: make sure to pipet the DNase I digestion mixture directly onto the membrane. DNase I digestion will not be complete if some of the mixture is retained on the wall or o-ring of the RNA binding column.
22. Let sit at room temperature for 15 minutes.
23. Add 350µL Buffer RW1. Centrifuge at maximum speed for 1 minute.
24. Aspirate and discard the filtrate and reuse the collection tube.
25. Add 500µL Wash Buffer RW. Centrifuge at maximum speed for 1 minute.
26. Aspirate and discard the filtrate and reuse the collection tube.

27. Repeat steps 25-26 for a second Wash Buffer RW wash step.
28. Centrifuge the empty RNA binding column for 2 minutes at maximum speed to dry the column matrix.

    Note: It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.
29. Transfer the RNA binding column into a 1.5mL microcentrifuge tube.
30. Add 50-70μL RNase-free water(Optional: pre-warm the water to 70–90°C will increase the RNA yield) directly onto the center of the membrane. Let sit at room temperature for 1 minute.
31. Centrifuge at maximum speed for 2 minutes.

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