
Version: 063015**PAX Blood miRNA Kit(For PAXgene Blood RNA Tube)****Cat#: RN51****❖ Kit Contents and Storage**

Kit Contents	Storage	50 Preps (RN5101)
Resuspension Buffer	RT	20 ml
Binding Buffer	RT	50 ml
WashSolution 1	RT	12 ml <i>Add indicated ethanol before first use</i>
WashSolution 2/3	RT	10 ml <i>Add indicated ethanol before first use</i>
RNase-free H ₂ O	RT	10 ml
DNase Buffer	- 20 °C	1.5 ml x 2
RNase free DNase I	- 20 °C	250 µl
Proteinase K (20mg / ml)	- 20 °C	20 mg x 1 tube
RNA Binding Columns	RT	50

Note: Proteinase K is a lyophilizate. Centrifuge a few seconds and reconstitute with 1 ml distilled water, aliquot solution. Store at -20 °C. Avoid repeated freezing and thawing.

All reagents, when store in indicated temperature, are stable for 9 months.

❖ Description

PAX Blood miRNA Kit is designed for isolation of total RNA >18 nucleotides (including miRNA) 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 miRNA 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 isopropanol, 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/miRNA. Genomic DNA is removed with an on-the-column DNase I digestion treatment. After three wash steps, purified RNA/miRNA is eluted with RNase-free water.

❖ **Materials and Equipment to be Supplied by User:**

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

❖ **Procedure**

Note:

⇒ **Before the first use, add the indicated amount of ethanol into Wash Solution 1 and Wash Solution 2/3 bottles, mix well, and mark the bottle with a check.**

⇒ **Heat the incubators or heat blocks to 55°C.**

1. Centrifuge the PAXgene® Blood RNA Tube for 10 minutes at 3,000-5,000 x g using a swing-out rotor.
2. Aspirate and discard the supernatant.
3. Add 4 mL RNase-free water. Vortex to completely resuspended the pellet.
4. Centrifuge the PAXgene® Blood RNA Tube for 10 minutes at 3,000-5,000 x g 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 350 µl Resuspension buffer. Vortex to completely resuspend

the pellet.

7. Transfer the sample into a new 1.5 ml microcentrifuge tube.
8. Add 300 μ l Binding Buffer and 20 μ L 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 a 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 equal volumes 100% isopropanol. Vortex to mix thoroughly.
13. Transfer up to 700 μ l mixture into a RNA binding column placed in a 2 ml 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 350 μ l Wash Solution 1.
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	45 μ l	450 μ l
RNase-free DNase I	5 μ l	50 μ l
Total volume	50 μ l	500 μ l

Important Notes:

- DNase I is very sensitive and prone to physical denaturing. Do not vortex the DNase I mixture. Mix gently 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 50 μ l 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 350 μ l Wash Solution 1. Centrifuge at maximum speed for 1 minute.

24. Aspirate and discard the filtrate and reuse the collection tube.

25. Add 500 μ l Wash Solution 2/3. 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 Solution 2/3 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 1.5 mL microcentrifuge tube.

30. Add 50-70 μ l 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.