CYRN51-CHANGYU PAX Blood miRNA Kit

Catalog number	Package
CYRN5101	50 preps

Storage: Resuspension Buffer, Binding Buffer, Wash Solution 1, Wash Solution 2/3, RNase-free H2O and RNA binding columns at room temperature; DNase Buffer, RNase-free DNase I and Proteinase K at -20 °C. All reagents are stable for 9 months when stored at the indicated temperatures. Proteinase K is a lyophilizate; centrifuge briefly, reconstitute with 1 ml distilled water, aliquot and store at -20 °C. Avoid repeated freezing and thawing.

Description

PAX Blood miRNA Kit is designed for isolation of total RNA larger than 18 nucleotides, including miRNA, from blood samples stored in preservation reagents and PAXgene Blood RNA Tubes. The procedure completely removes contaminants and enzyme inhibitors and produces high-quality RNA suitable for applications such as RT-PCR. Samples are removed from the preservation reagent; for blood stored in PAXgene Blood RNA Tubes, cells are collected by centrifugation, washed and lysed in an optimized buffer containing Proteinase K. The sample is centrifuged to remove cell debris and particulates. After adjusting binding conditions with isopropanol, the sample is loaded onto an RNA binding column; a brief centrifugation or vacuum step passes the sample through the matrix, which binds RNA/miRNA. Genomic DNA is removed by on-column DNase I digestion, and after three washes purified RNA/miRNA is eluted with RNase-free water.

Kit components

Component	Storage	50 preps (CYRN5101)
Resuspension Buffer	15-25 °C	20 ml
Binding Buffer	15-25 °C	50 ml
Wash Solution 1	15-25 °C	12 ml (add ethanol before first use)
Wash Solution 2/3	15-25 °C	10 ml (add ethanol before first use)
RNase-free H2O	15-25 °C	10 ml
DNase Buffer	-20 °C	1.5 ml × 2
RNase-free DNase I	-20 °C	250 µl
Proteinase K (20 mg/ml after reconstitution)	-20 °C	20 mg × 1 tube
RNA binding columns	15-25 °C	50

Materials and equipment to be supplied by the user

1. Microcentrifuge capable of 13,000 × 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 incubator or heat block capable of 55 °C and 65 °C.
7. Centrifuge with a swing-bucket rotor capable of 5,500 × g.

❖ Procedure

Before first use, add the indicated amount of ethanol to Wash Solution 1 and Wash Solution 2/3, mix well and tick the bottles. Heat the incubator or heat block to 55 °C.

1. Centrifuge the PAXgene Blood RNA Tube at 3,000-5,000 × g for 10 minutes using a swing-out rotor.

2. Aspirate and discard the supernatant.

3. Add 4 ml of RNase-free water and vortex to resuspend the pellet completely.

4. Centrifuge the PAXgene Blood RNA Tube at 3,000-5,000 × g for 10 minutes using a swing-out rotor.

5. Aspirate and discard the supernatant. Incomplete removal of the supernatant reduces lysis efficiency and dilutes the lysate, reducing RNA yield.

6. Add 350 µl of Resuspension Buffer and vortex to resuspend the pellet completely.

7. Transfer the sample into a new 1.5 ml microcentrifuge tube.

8. Add 300 µl of Binding Buffer and 20 µl of Proteinase K Solution and vortex for 5 seconds.

9. Incubate at 55 °C for 10 minutes in a shaking incubator.

10. Optional: pass the lysate at least 5 times through a 20-gauge (0.9 mm) needle fitted to a syringe, or homogenize with an electronic tissue homogenizer. This shears genomic DNA, reduces lysate viscosity and increases yield.

11. Centrifuge the homogenized lysate at 13,000 rpm for 3 minutes and transfer the supernatant to a new centrifuge tube.

12. Add an equal volume of 100% isopropanol and vortex to mix thoroughly.

13. Transfer up to 700 µl of the mixture into an 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 all of the remaining sample has been transferred to the RNA binding column.

17. Add 350 µl of 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 RNA binding column, prepare the DNase I digestion reaction mix: 45 µl of DNase I Buffer and 5 µl of RNase-free DNase I per prep (for 10 preps: 450 µl and 50 µl; total volume 50 µl per prep). Important: DNase I is very sensitive and prone to physical denaturation; do not vortex the mixture, mix gently by inversion. Prepare the DNase I mix fresh immediately before RNA isolation. Standard DNase buffers are not compatible with on-membrane DNase I digestion; other buffers may affect RNA binding to the matrix and reduce yield and purity. Perform all steps at room temperature, working quickly but carefully.

21. Pipette 50 µl of DNase I digestion reaction mix directly onto the centre of the RNA binding column membrane. Make sure the mixture is applied directly to the membrane; digestion will be incomplete if some of the mixture remains on the wall or O-ring of the column.

22. Leave at room temperature for 15 minutes.

23. Add 350 µl of Wash Solution 1 and centrifuge at maximum speed for 1 minute.

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

25. Add 500 µl of Wash Solution 2/3 and 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.

28. Centrifuge the empty RNA binding column at maximum speed for 2 minutes to dry the column matrix. Drying the membrane before elution is important; residual ethanol may interfere with downstream applications.

29. Transfer the RNA binding column into a 1.5 ml microcentrifuge tube.

30. Add 50-70 µl of RNase-free water directly onto the centre of the membrane (optional: prewarm the water to 70-90 °C to increase RNA yield) and leave at room temperature for 1 minute.

31. Centrifuge at maximum speed for 2 minutes.

This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.