CYRN50-CHANGYU RNAstore Blood RNA Kit

Catalog number	Package
CYRN5001	50 preps

Storage: Lysis Solution at 4 °C; Sodium Acetate Buffer, Buffer RW1, Wash Buffer RW, RNase-free H2O, RNAfixer (RNAlater) solution and RNA binding columns at room temperature; DNase Buffer and RNase-free DNase I at -20 °C. All reagents are stable for 9 months when stored at the indicated temperatures.

Description

RNAstore Blood RNA Kit is designed for isolation of total RNA from blood samples stored in RNAfixer (same as RNAlater) solution. Blood samples can be processed immediately, or stored in RNAfixer solution (provided with the kit) for a few days at ambient temperature or for prolonged periods at -20 °C before RNA extraction. The RNA isolation procedure has two parts: (1) cell lysis in a guanidinium-based solution and initial purification of RNA by phenol/chloroform extraction; (2) final RNA purification by solid-phase extraction on a glass fiber filter. This procedure completely removes contaminants and enzyme inhibitors and produces high-quality RNA suitable for applications such as qRT-PCR.

Kit components

Component	Storage	50 preps (CYRN5001)
Lysis Solution	4 °C	40 ml
Sodium Acetate Buffer	15-25 °C	5 ml
Buffer RW1	15-25 °C	40 ml
Wash Buffer RW	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
RNAfixer (RNAlater) solution	15-25 °C	70 ml
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% ethanol and water-saturated phenol:chloroform (5:1).
3. RNase-free filter pipette tips.
4. Blood collection tubes (recommended anticoagulants: potassium EDTA or sodium EDTA).
5. 1.5 or 2.0 ml microcentrifuge tubes.
6. Shaking incubator or heat block capable of 75-90 °C.

❖ Procedure

Before first use, add the indicated amount of ethanol to Wash Buffer RW, mix well and tick the bottle. Heat the incubator or heat block to 75 °C and prewarm an aliquot of RNase-free H2O to increase yield.

1. Collect blood samples by standard methods in tubes containing anticoagulant. Potassium or sodium EDTA are recommended; heparin and citrate are also compatible. Other anticoagulants have not been tested.

2. (Optional) Add 300-500 µl of blood to 1.3 ml of RNAfixer (RNAlater) solution. 2a. Mix the blood sample by gently inverting the collection tube several times. 2b. Add 300-500 µl of anticoagulated blood to 1.3 ml of RNAfixer in a 2 ml microcentrifuge tube (not included) and mix thoroughly by inverting several times. Once mixed with RNAfixer, a sample can be stored for up to 3 days at ambient temperature; longer storage at ambient temperature gradually reduces RNA yield and quality. Samples mixed with RNAfixer can also be stored long-term at -20 °C.

3. For samples in RNAfixer solution: centrifuge and remove the supernatant. 3a. Centrifuge for 1 minute in a microcentrifuge. Blood cells and plasma proteins form a large brown or reddish-brown pellet that may smear up the side of the tube; the supernatant may be pale pink, brown or colorless (often turbid). 3b. Remove and discard the supernatant by aspiration or pouring. When aspirating, remove all fluid, including the more turbid portion directly above the pellet, which may contain white particulate matter; this is not the buffy coat seen in untreated whole blood after centrifugation. If pouring, tap the rim of the inverted tube against a paper towel to remove residual fluid. Remove any fluid from inside the tube cap.

4. Lyse blood cells in 800 µl of Lysis Solution and 50 µl of Sodium Acetate Buffer. 4a. If the blood sample was not stored in RNAfixer, mix by gently inverting the collection tube several times. 4b. Add 800 µl of Lysis Solution and 50 µl of Sodium Acetate Buffer to 300-500 µl of anticoagulated whole blood in a 2 ml microcentrifuge tube. 4c. Vortex vigorously to lyse the blood cells and invert the tube to ensure homogeneity. Samples stabilized in RNAfixer require more vigorous vortexing to resuspend and lyse the cells.

5. Extract with 500 µl of water-saturated phenol:chloroform (5:1). 5a. Add 500 µl of water-saturated phenol:chloroform (5:1) to the cell lysate and shake vigorously or vortex for 30 seconds. If adding 500 µl would make the tube too full to mix adequately, use as little as 250 µl or split the sample into two tubes. 5b. Leave at room temperature for 5 minutes. 5c. Centrifuge at room temperature for 1 minute to separate the aqueous and organic phases; the aqueous phase may be cloudy or clear.

6. Recover the aqueous phase into a fresh 2 ml tube. Transfer the upper aqueous phase containing RNA to a new 2 ml tube (not provided). If the sample was split for phenol:chloroform extraction, combine the aqueous phases into one tube. The aqueous phase volume is typically about 1-1.2 ml. Avoid transferring colored material from the lower organic phase, which contains heme and proteins; discard the lower phase. If the aqueous phase volume is less than 800 µl, see Appendix I.

7. Add 600 µl of 100% ethanol to each sample and mix well. Add 600 µl (about one-half volume) of 100% ethanol to each tube of aqueous phase and vortex briefly but thoroughly. If desired, centrifuge very briefly (about 1 second) to collect fluid from around the lid.

8. Transfer up to 700 µl of the mixture into an RNA binding column placed in a 2 ml collection tube (provided).

9. Centrifuge at maximum speed for 1 minute.

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

11. Repeat steps 8-10 until all of the remaining sample has been transferred to the RNA binding column.

12. Add 350 µl of Buffer RW1.

13. Centrifuge at maximum speed for 1 minute.

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

15. 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.

16. 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.

17. Leave at room temperature for 15 minutes.

18. Add 350 µl of Buffer RW1 and centrifuge at maximum speed for 1 minute.

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

20. Add 500 µl of Wash Buffer RW and centrifuge at maximum speed for 1 minute.

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

22. Repeat step 20 for a second Wash Buffer RW wash.

23. 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.

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

25. 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.

26. Centrifuge at maximum speed for 2 minutes.

❖ Appendix I: Difficulty recovering the aqueous phase after organic extraction

Occasionally after the phenol:chloroform extraction in step 5, the aqueous phase volume is less than about 1 ml, the interphase is unusually thick, and it is difficult to remove the aqueous phase without contaminating it with organic-phase material. This may be caused by suboptimal mixing during phenol:chloroform extraction when the tube is too full, and may also be seen in samples with unusually high white blood cell content.

1. After the centrifugation step of phenol:chloroform extraction, remove as much aqueous phase as possible to a new tube and determine its approximate volume.

2. Add additional Lysis Solution to the organic phase to bring the total aqueous phase volume to 1.2 ml. For example, if 800 µl of aqueous phase was initially recovered, add 400 µl of additional Lysis Solution to the tube containing the organic phase.

3. Mix the Lysis Solution and organic phase by brief vigorous vortexing, then centrifuge for 1 minute at maximum speed to separate the phases.

4. Remove the aqueous phase and pool it with the aqueous phase recovered initially.

5. Add one-half volume of 100% ethanol, mix well and continue the procedure at step 8.

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