Version: 063015

RNAstore Blood RNA Kit(For RNAlater Preserved Blood)

Cat#：RN50

❖ Kit Contents and Storage

Kit Contents	Storage	50 Preps (RN5001)
Lysis Solution	4 °C	40 ml
Sodium Acetate Buffer	RT	5 ml
Buffer RW1	RT	40 ml
Wash Buffer RW	RT	10 ml *Add indicated ethanol before first use*
RNase-free H_2O	RT	10 ml
DNase Buffer	－20 °C	1.5 ml x 2
RNase free DNase I	－20 °C	250 µl
RNAfixer(RNAlater) solution	RT	70 ml
RNA Binding Columns	RT	50

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

❖ 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 they can be stored in RNAfixer Solution(provided with the kit) for a few days at ambient temperature or for prolonged periods at -20°C prior to RNA extraction. The Kit RNA isolation procedure consists of two parts:

1. Cell lysis in a guanidinium-based solution and initial purification of the 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 producing high-quality RNA. RNA purified using the Kit is ready

for applications such as qRT-PCR.

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

1. Microcentrifuge capable of 13,000 x g
2. 100% ethanol, water-saturated-Phenol: Chloroform(5:1)
3. RNase-free filter pipette tips
4. Blood collection tubes (recommended anticoagulant: potassium EDTA or sodium EDTA)
5. 1.5 or 2.0 ml microcentrifuge tubes
6. Shaking incubators or heat blocks capable of 75°C-90°C

❖ **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 75°C. Heat an aliquot of the RNase-free H_2O to increase yield .**

1. Collect blood samples using standard methods

   Collect blood samples according to standard procedures in tubes containing anticoagulant. Potassium or sodium EDTA are the recommended anticoagulants, but heparin and citrate are also compatible with the procedure. Other anticoagulants have not been tested.

2. (Optional) Add300–500 µl blood to 1.3 mL RNAfixer (RNAlater) Solution

2a. Mix blood sample by gently inverting the collection tube several times..

2b. Add 300–500µl anticoagulated blood to 1.3 ml RNAfixer in a 2 ml microfuge tube (not included in the kit). Mix thoroughly by inverting the tube several times.

   Once a sample is mixed with RNAfixer, it can be stored for up to 3 days at ambient temperature. Storing RNAfixer treated samples for longer periods at ambient temperature will result in a gradual

decrease in RNA yield and quality.

Samples mixed with RNAfixer can also be stored at –20°C for long-term Storage.

3. Samples in RNAfixer Solution: centrifuge and remove the supernatant

3a. Centrifuge sample for 1 min in a microcentrifuge. The blood cells and plasma proteins will form a large brown or reddish-brown pellet which may smear upward along the side of the tube, and the supernatant may be pale pink, brown, or colorless (but it is often turbid).

3b. Remove and discard the supernatant by aspiration or pouring.

- When aspirating the supernatant, be sure to thoroughly remove all of the fluid, including the portion directly above the cell pellet, which may be more turbid, and which may contain some white particulate matter. Note, this material is not the “buffy coat” fraction seen in untreated whole blood after centrifugation.
- If the supernatant is removed by pouring, tap the rim of the inverted tube gently against a paper towel to remove all residual fluid.
- Remove any fluid from inside the tube cap.

4. Lyse blood cells in 800 µl Lysis Solution and 50 µl Sodium Acetate Buffer.

4a. If the blood sample was not stored in RNAfixer Solution, mix by gently inverting the collection tube several times.

4b. Add 800 µl Lysis Solution and 50 µl Sodium Acetate Buffer to 300–500 µl anticoagulated whole blood in a 2 ml microfuge tube.

4c. Vortex vigorously to lyse the blood cells. Invert the tube to be sure the solution is homogenous. Samples that were stabilized in RNAfixer will require more vigorous vortexing to resuspend and lyse

the cells.

5. Extract with 500 µl 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 sec. Note: If addition of 500 µL of Phenol:Chloroform would cause the tube to be too full to permit adequate mixing, you can use as little as 250 µl of Phenol:Chloroform. Or split it into 2 microtubes to extract.

5b. Store the mixture at room temp for 5 min.

5c. Centrifuge at room temp for 1 min to separate the aqueous and organic phases. The aqueous phase may appear cloudy or clear after centrifugation.

6. Recover the aqueous phase in a fresh 2 ml tubeds.

- Transfer the aqueous (upper) phase containing the RNA to a new 2 ml tube (not provided in the kit). If samples were split for the Phenol:Chloroform extraction, collect the aqueous phases into a single tube..
- Typically the aqueous phase volume is ~1–1.2 ml.
- Avoid transferring the colored material from the organic (lower) phase, which contains heme and proteins. Discard the lower phase.

Note: If the aqueous phase volume is less than 800 µl, see Affix I. Difficulty Recovering Aqueous Phase after Organic Extraction on page 5**.**

7. Add 600 µl of 100% ethanol to each sample, mix well.

- To each tube of aqueous phase recovered after the Phenol: Chloroform extraction, add 600 µl (~one-half volume) of 100% ethanol, and vortex briefly but thoroughly.
- If desired, the tube may then be centrifuged very briefly (~1 sec) to collect the fluid from around the lid of the tube.

8. Transfer up to 700 µl mixture into a 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 the remaining sample has been transferred to RNA binding column.
12. Add 350 µl Buffer RW1.
13. Centrifuge at maximum speed for 1 minute.
14. Aspirate and discard the filtrate and reuse the collection tube.
15. 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 µl	450 µl
RNase-free DNase I	5 µl	50 µl
Total volume	50 µl	500 µl

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.

16. Pipet 50 µl 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
17. Let sit at room temperature for 15 minutes.
18. Add 350 µl Buffer RW1. Centrifuge at maximum speed for 1 minute.

19. Aspirate and discard the filtrate and reuse the collection tube.
20. Add 500 µl Wash Buffer RW. Centrifuge at maximum speed for 1 minute.
21. Aspirate and discard the filtrate and reuse the collection tube.
22. Repeat steps 25-26 for a second Wash Buffer RW wash step.
23. 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.
24. Transfer the RNA binding column into a 1.5 mL microcentrifuge tube.
25. Add 50-70 µl 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.
26. Centrifuge at maximum speed for 2 minutes.

❖ **Affix I: Difficulty Recovering Aqueous Phase after Organic Extraction**

Occasionally after the Phenol:Chloroform extraction step 5, the volume of the aqueous phase is less than ~1 mL, the interphase is unusually thick, and it is difficult to remove the aqueous phase without contaminating it with material from the organic phase. This may be caused by suboptimal mixing of the sample during the phenol:chloroform extraction step if the tube is too full to thoroughly mix the samples. This may also be seen in samples with an unusually high WBC content.

To recover the RNA from samples with less than ~0.8 ml of aqueous phase:

1. After the centrifugation step of the Phenol:Chloroform extraction, remove as much of the 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 in the acid phenol chloroform extraction, add 400 µl additional Lysis Solution to the tube with the organic phase.

3. Mix the Lysis Solution + organic phase by brief vigorous vortexing, then centrifuge for 1 min 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