EZ-press Whole Blood RNA Purification Kit

Catalog No.: B0006

Description

The *EZBioscience*® EZ-press Whole Blood RNA Purification Kit provides a simple, reliable, and rapid method for isolating RNA from fresh or frozen anti-coagulated blood, without toxic substances such as phenol or chloroform. The purified RNA is suitable for all common molecular biological applications directly: PCR, qPCR, RNA sequencing, and membrane hybridization (e.g., Nouthern blot analysis). Whole blood samples are first lysed and homogenized in a strong denaturant and phenol containing buffer, which immediately inactivates RNases to ensure isolation of intact RNA. After homogenization, chloroform is mixed with the lysate and incubated for 3 minutes. And then the mixture is centrifuged for phase separation; the upper-layer supernatant is collected and and then passed through the DNA Removing Spin Column which traps the DNA on the column, while RNA can pass through the column with the flow-through. Then ethanol is added to the RNA containing flow-through. The mixture is then passed through the RNA binding Spin Column to which the RNA binds. Impurities are effectively removed by subsequent washing. The purified total RNA is then eluted with Elution Buffer and may be used in a variety of downstream applications.

Components

Components	B0006 (100 Preps)
RCL Buffer	60 ml
Lysis Buffer	55 ml
Wash Buffer*	13 ml
Elution Buffer	25 ml
DNA Removing Spin Columns (with Collection Tubes)	100 preps
Spin Columns for RNA (with Collection Tubes)	100 preps

*Before using for the first time, add 52 ml of 100% ethanol to the Wash Buffer.

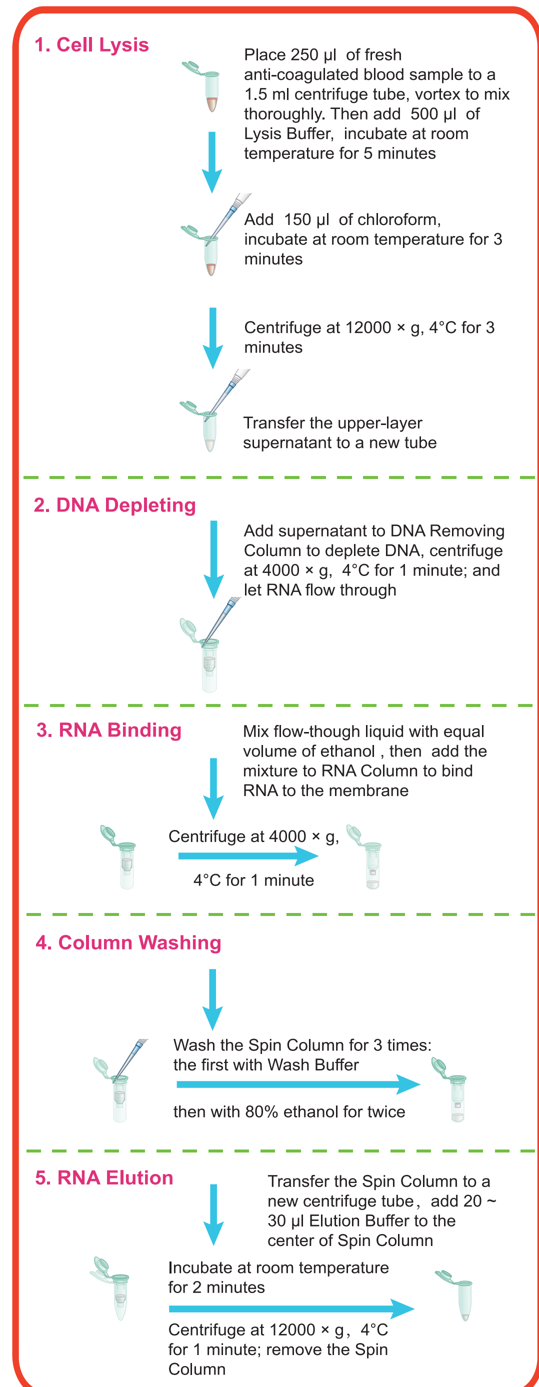
Storage

Store the RCL Buffer and Lysis Buffer at 2 ~ 8°C upon reception, protect from light. Store other components at room temperature (Divide the Elution Buffer into 3 aliquots upon reception and store at room temperature). When using these buffers, be careful to avoid of contamination.

Notice

1. When collecting blood samples, citric acid (sodium) or EDTA is recommended as the anti-coagulant, but heparin (sodium) is not recommended. Otherwise, the experiment results will be significantly affected.
2. Wash Buffer must be mixed with 52 ml 100% ethanol before it can be used.
3. Before the experiment, 90 ml 80% ethanol should be prepared with sterilized water for column washing.
4. RNA purification should be carried out at room temperature and not be placed on ice.

Experimental Procedure Overview:



Protocol

Sample Lysis

1. Place 250 ul whole blood in a 1.5 ml centrifuge tube(If the volume of whole blood is less than 250 ul, add RCL to 250 ul), add 500 μl of Lysis Buffer, use vortex oscillator to vortex 10 seconds to mix thoroughly.
2. After mixed, Incubate the mixture at room temperature for 5 minutes to lysis thoroughly.
3. Add 150 μl chloroform to the lysates, mixed by vortex oscillator, and Incubate at room temperature for 3 minutes.
4. Centrifuge at 12000 × g, 4°C for 3 minutes. Then the mixture is separated into three phases. Transfer the upper-layer supernatant (about 300 μl) to a new RNase free 1.5 ml centrifuge tube (be careful to avoid disturbing the middle or bottom layer).

DNA Depleting

5. Transfer the homogenized lysate of step 4 to the DNA Removing Spin Column, centrifuge at 4000 × g, 4°C for 1 minute.

RNA Binding

6. Adding equal volume of 100% ethanol to flow-through liquid containing RNA, use Pipette to pipette up and down for 10 times to mix thoroughly. Transfer the mixture to the Spin Column for RNA, centrifuge at 4000 × g, 4°C for 1 minute. Pour out the liquid in the collection tube, and then place the collection tube on absorbent paper to suck the liquid from the orifice of the tube. Put the Spin Column back into the collection tube.

Column Washing

7. Add 500 μl of Wash Buffer to the Spin column, centrifuge at 12000 × g, 4°C for 1 minute. Pour off the liquid and eliminate the residual liquid using towel paper. Place the empty column back on the collection tube.
8. Add 300 μl of 80% ethanol to the column, centrifuge at 12000 × g, 4°C for 1 minute. And then add 300 μl of 80% ethanol to the column, centrifuge at 12000 × g, 4°C for 1 minute (Important: be careful to avoid the bottom of the column touching with the flow-through when taking the column out from the collection tube, Otherwise the wash buffer will contaminate the RNA. If the liquid accidentally touched the bottom of the Spin Column, discard the liquid and place the column back to the collection tube, centrifuge for 1 minute to thoroughly remove the liquid on the column).
9. Don't need to pour off the liquid, directly transfer the column to a new 1.5 ml centrifuge tube, Open the lid and keep in the air for 2 minutes.

RNA Elution

10. Add 20 ~ 30 μl of Elution Buffer to the center of the column, and incubate at room temperature for 2 minutes.
11. Centrifuge at 12000 × g, 4°C for 1 minute. Discard the column, determine the RNA concentration, do the following experiment with the purified RNA, or store the RNA at -20°C until needed (For long-term preservation, it is recommended to place it at -80°C).

Representative Results

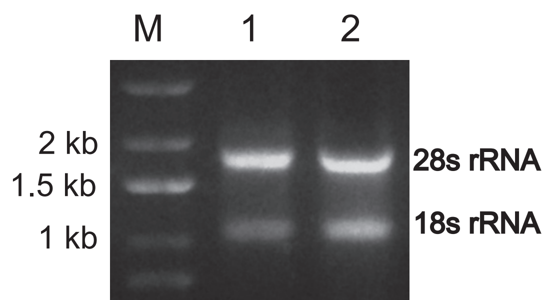


Figure 1. RNA isolated from 100 μl and 200 μl whole blood using *EZ*Bioscience® EZ-press Whole Blood RNA Purification Kit. RNA was eluted by 50 μl Elution Buffer, and 5 μl was loaded each lane. M: 250bp DNA Ladder; Lane 1: RNA isolated from 100 μl whole blood; Lane 2: RNA isolated from 200 μl whole blood.

Trouble Shooting

1. The RNA purity is not good enough.

a. Check whether ethanol is properly added to the reagent used, whether it is contaminated. Do the experiment with new products, and compare the results of the two groups. It is suggested that after the kit is opened, divide each buffer into 3 aliquots (which can be packed in 15 ml centrifugal tube). It should be strictly operated in accordance with the manual to prevent cross-contamination.

b. After centrifugation, the liquid in the collection tubes should be poured out and absorbent paper should be used to absorb the remaining liquid at the collection tube orifice. Operate strictly in the same way after each washing step.

c. Wash the column with Wash Buffer first, and then with 80% ethanol for twice. Be careful to avoid the lower side of the column touching with the flow-through when taking the column out from the collection tube after each wash step. Otherwise the wash buffer will contaminate the RNA. If the liquid accidentally touched the bottom of the Spin Column, discard the liquid and place the column back to the collection tube, centrifuge for 1 minute to thoroughly remove the liquid on the column.