RNApure Blood Kit

Blood RNA Extraction Kit

Project No. R666034 (50 preps)
Storage conditions: room temperature (15-30° C)

Product content

individual parts making up a compound	R666034 50 preps
Buffer RBL (10×)	60 ml
Buffer RL	35 ml
Buffer RW1	40 ml
Buffer RW2 (concentrate)	11 ml
RNase-Free Water	10 ml
Spin Columns FL with Collection Tubes	50
Spin Columns RM with Collection Tubes	50
RNase-Free Centrifuge Tubes (1.5 ml)	50

Product Introduction

This kit is suitable for extracting total RNA from fresh whole blood (blood samples treated with anticoagulants such as citrate, EDTA or heparin). It can process up to 1.5 ml of whole blood and elute high-purity RNA with molecular weight greater than 200 bp, and multiple samples can be processed at the same time in less than 1 hour. The product does not require ultracentrifugation for CsCl purification and LiCl or ethanol precipitation, and does not contain toxic solvents such as phenol or chloroform, etc. The purified RNA effectively removes enzyme inhibitors and contaminants such as hemoglobin and heparin, and can be used in a variety of routine molecular biology experiments, such as RT-PCR, Northern Blot, Dot Blot, and in vitro translation, etc. The product can also be used in a wide range of molecular biology experiments.

Self-contained reagents: β-mercaptoethanol, 70% ethanol (prepared with RNase-free water), anhydrous ethanol.

Pre-experiment Preparation and Important Notes

1. To prevent RNase contamination, attention should be paid to the following aspects:
1) Use RNase-free plastics and tips to avoid cross-contamination.
(2) Glassware should be dry baked at a high temperature of 180 ℃ for 4 hours before use, plasticware can be immersed in 0.5M NaOH for 10 minutes, rinsed thoroughly with water and autoclaved.
3) RNase-free water should be used to prepare the solution.
(4) Operators wear disposable masks and gloves, and change gloves diligently during the experiment.
2. Avoid repeated freezing and thawing of samples, as this may affect the rate and quality of RNA extraction. Samples can be stored in Buffer RL at -70° C for one month.
3. Before use, check Buffer RL for crystallization or precipitation, which can be re-dissolved in a water bath at 56° C. Add β-mercaptoethanol to Buffer RL before use to a final concentration of 1%. For example, add 10 μl of β-mercaptoethanol to 1 ml of Buffer RL. Buffer RL with β-mercaptoethanol can be stored for 1 month at room temperature.
4. Anhydrous ethanol should be added to Buffer RW2 according to the instructions on the label of the reagent bottle before first use.
5. This kit cannot be used for RNA extraction from frozen blood samples with anticoagulant added.
6. 10×Buffer RBL should be diluted 10-fold with RNase-free water before use, and stored at 2-8° C after dilution.
7. If downstream experiments are very sensitive to DNA, it is recommended that RNA be treated with RNase-free DNase I.
8. All centrifugation steps are carried out at room temperature unless otherwise indicated, and all steps are performed quickly.

Operation steps

1. To 0.5-1.5 ml of fresh anticoagulated whole blood sample, add 5 times the volume of 1 x Buffer RBL (dilute 10 x Buffer RBL 10-fold with RNase-free water before use) and gently vortex or invert. Incubate on ice for 10-15 minutes, mixing twice during incubation.
Note: Turbid suspensions will become clear during incubation, proving that the erythrocytes are lysed, and the incubation time can be extended to 20 minutes if necessary.
2. Centrifuge at 2,100 rpm (~400 × g) for 10 minutes at 4° C and carefully aspirate the supernatant.
3. Add 1×Buffer RBL (please dilute 10×Buffer RBL 10-fold with RNase-free water before use) of twice the volume of the blood sample to the above precipitate, vortex gently, and resuspend the precipitate thoroughly.

4. Centrifuge at 2,100 rpm for 10 minutes at 4° C and carefully and thoroughly aspirate the supernatant.
Note: The supernatant must be completely removed in this step or it will affect the cleavage resulting in decreased RNA yield.
5. Add Buffer RL to the precipitate (check that β-mercaptoethanol has been added before use), add 600 μl Buffer RL to 0.5-1.5 ml of blood sample or 350 μl Buffer RL to less than 0.5 ml of blood sample and mix well.
6. Transfer the resulting liquid to a filter column (Spin Columns FL) that has been loaded into a collection tube, centrifuge at 12,000 rpm (~13,400 x g) for 2 minutes, collect the filtrate, and discard the filter column.
7. To the resulting filtrate, add 1x (600 μl or 350 μl) of 70% ethanol (prepared in RNase-free water) and mix well.
Note: The addition of ethanol may produce a precipitate that will not affect subsequent experiments.
8. Add all of the solution obtained in the previous step to the Spin Columns RM in the collection tube. If the solution cannot be added all at once, it can be transferred several times. centrifuge at 12,000 rpm for 15 seconds, pour off the waste liquid in the collection tube and put the column back into the collection tube.
9. Add 700 μl of Buffer RW1 to the adsorbent column, centrifuge at 12,000 rpm for 15 seconds, pour off the waste liquid from the collection tube, and place the column back into the collection tube.
OPTIONAL STEP: If RNA experiments that are very sensitive to trace DNA are to be performed, replace step 9 with the following step.
1) Add 350 μl of Buffer RW1 to the adsorbent column, centrifuge at 12,000 rpm for 15 seconds, pour out the waste liquid from the collection tube, and put the adsorbent column back into the collection tube.
(2) Preparation of DNase I mixture: Take 70 μl of Reaction Buffer and 10 μl of DNase I stock solution, mix gently to form a final volume of 80 μl of reaction solution.
Note: The above system is configured according to our product DNase I (CW2090S), please refer to the corresponding manual for other products.
3) Add 80 μl of prepared DNase I reaction solution directly to the adsorption column and incubate at 20-30° C for 15 minutes.
4) Add 350 μl Buffer RW1 to the adsorption column, centrifuge at 12,000 rpm for 15 seconds, pour off the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
10. Add 500 μl of Buffer RW2 to the adsorbent column (check that anhydrous ethanol has been added before use), centrifuge at 12,000 rpm for 15 seconds, pour off the waste liquid from the collection tube, and put the adsorbent column back into the collection tube.
11. Repeat step 10.
12. Centrifuge the column at 12,000 rpm for 2 minutes and pour off the waste liquid from the collection tube. Leave the column at room temperature for several minutes to dry thoroughly.

Note: The purpose of this step is to remove residual ethanol from the adsorption column, which can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).

13. Place the adsorbent column in a new RNase-free centrifuge tube, add 30-50 μl of RNase-Free Water to the middle of the adsorbent column, leave at room temperature for 1 minute, centrifuge at 12,000 rpm for 1 minute, collect the RNA solution, and store the RNA at -70° C to prevent degradation.

Attention:

(1) The volume of RNase-Free Wate should not be less than 30 μl, too small a volume affects the recovery rate.

2) If you want to increase the RNA yield, repeat step 13 with 30-50 μl of fresh RNase-Free Water.

3) If the RNA concentration is to be increased, the resulting solution can be reintroduced into the adsorption column and step 13 repeated.