Quick Protocol

RNJia Phenol-free PB Kit

(With RBC Lysis Buffer)

RNJia Phenol-free PB Kit

(No RBC Lysis Buffer)

RNA and total RNA isolation based on silica technology

- MiniPrep

For RNA Isolation from

PBMC (Peripheral Blood Mononuclear Cell)

Whole Blood

WBC (White Blood Cell)

Kit Content

Component	100 preps
RLB	2 x 35 ml
TWB1 (concentrate)	2 x 16 ml
TWB2 (concentrate)	2 x 15 ml
Nuclease-free RBC Lysis buffer Just included in RNJia Phenol-free PB Kit, (With RBC Lysis Buffer)	2×375 ml
Nuclease-free Water	10 ml
Micro Column	100
Collection Tube	300

Recommended Starting Material

To reach optimized results it is better to follow as listed below. The size of the recommended starting material to use with determined RLB volumes is written in Table 1.

Table 1. Appropriate sample size and amount of RLB

Whole blood (ml)	Number of WBCs	Amount of RLB (µl)
Up to 0.5	Up to 2 x 10^6	400
0.5 to 1.5	2 x 10^6 to 1 x 10^7	700

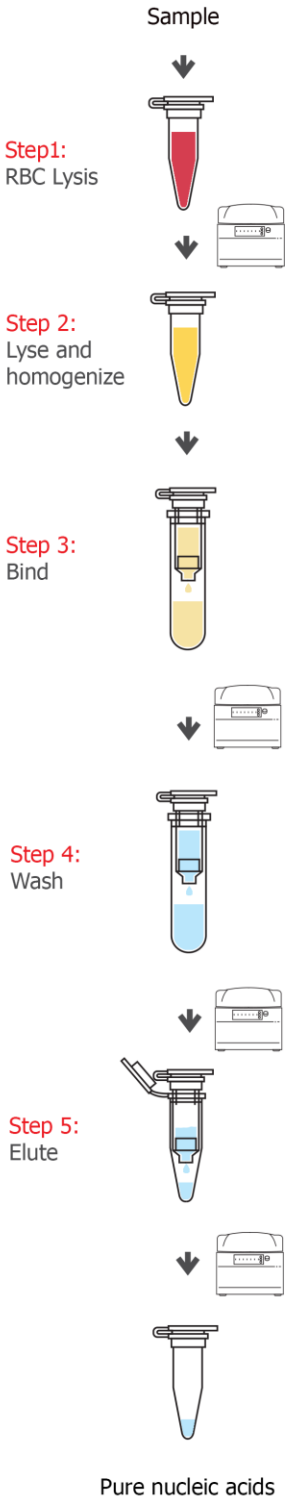
Washing Buffer Preparation

Before the first use, add appropriate amount of ethanol (96-100%) to each washing buffer tube, then mix thoroughly to prepare washing buffer, refer to Table 2. Do not forget to tick the check box on the bottle label to indicate that ethanol has been added. Before each use mix reconstituted buffer by shaking. Storing at room temperature

Table 2: Washing buffer preparation

Buffer Name	Concentrated Volume	Amount of Ethanol	Final Volume
TWB1	16ml	24ml	40ml
TWB2	15ml	45ml	60ml

Procedure of silica-based RNA isolation in quick look



Protocols

Phenol-Chloroform Free Based Protocols

Protocol: Isolation of RNA (PBMC, WBC, and Whole blood)

Sample Type: PBMC (Peripheral Blood Mononuclear Cell), WBC (White Blood Cell), and Whole blood

Some tips to know:

- All steps, before applying the sample to Micro Column, are carried out on the ice.

Before using the protocol for the first time, add 2-Mercaptoethanol to RLB, 1% of RLB volume. It is better to calculate it every time you test and add 1% of 2ME to it for higher efficiency. It is 693 µl of RLB and 7 µl of 2ME for each sample test. 2-Mercaptoethanol is commercially available and due to safety issues not provided in the kit.

Attention: It is recommended to prepare it freshly. It is not stable for more than 2 weeks.

- If RLB forms a precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage conditions and will not influence the efficiency of the buffer
- Do Not forget to add the appropriate amount of ethanol (molecular biology grade, 96–100%) to TWB1 and TWB2 as indicated on the bottle, before using them for the first time. Refer to the washing buffer preparation section.
- If working with RNA for the first time, read Appendix 1 carefully.

Process

1. Collect 0.5 to 1.5 ml of blood into EDTA tubes. Add Five volumes of Nuclease-free RBC Lysis Buffer. Vortex the tube for 15s and incubate at 4 ºc for 20 min or incubate at freezer -20 ºc for 10 min.
2. Pulse vortex and five times invert every 3 min during incubation to intersperse the sample.
3. Collect the WBCs by centrifugation at 400 x g for 10 min at 4ºc.
4. Discard the supernatant; add two volumes of Nuclease-free RBC Lysis Buffer to the pellet, vortex until the pellet is dissolved completely.
5. Collect the WBCs by centrifugation at 400 g for 10 min at 4ºc.

   Note: To remove erythrocyte cells, you can use 1 ml of peripheral blood into the Falcon 15 and add 5 ml of RBC lysate to it, vortex for 15 seconds, place in the freezer for 15

minutes, and 10 times invert the Falcon every 3 minutes. then centrifuge at 1500 rpm for 10 minutes

After that, discard the supernatant and add 1 ml of RBC to the falcon Pipet it well and then transfers all the contents to a sterile 1.5 vial.

centrifugation at 1750 rpm for 10 minutes

6. Discard the supernatant. Disrupt the cells by adding RLB (prepared with 2-βME). Loosen the cell pellet thoroughly by flicking the tube. Add the appropriate volume of RLB vortex or pipet to mix. for example, for the volume of up to 500 µl of blood, add the amount of 400 µl of RLB and for the volume of the blood sample up to 1.5 ml/liter, add the amount of 700 µl of RLB.

After adding the appropriate amount of RLB, use a Micro pestle followed by a homogenizer or syringe needle to homogenize the sample for 1min.

Note: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced RNA yields.

Note: For previously isolated PBMCs and WBCs, start from step 6.

7. Centrifuge the lysate for 3 min at 15000 rpm. Carefully remove the supernatant by pipetting, and transfer it to a new RNase-free microcentrifuge tube.
8. Add one volume of 70% Cold Ethanol to the homogenized lysate, and mix well with a calm pipette.

   Note: When purifying RNA from isolated cells, precipitates may be visible after the addition of ethanol. This does not affect the procedure.
9. Transfer up to 700 µl of the sample, including any precipitate that may have formed, to a Micro Column placed in a 2ml collection tube (supplied in the kit box). Close the lid gently, and centrifuge for 15s at 12000 rpm. Discard the flow-through. Reuse the collection tube in step 10
10. add the remainder of the sample to the column and centrifuge for 15s at 12000 rpm Discard the flow-through new collection
11. Add 700 µl TWB1 to the Micro Column. Centrifuge for 15s at 12000 rpm at room temperature. Discard the flow-through.
12. Add 600 µl TWB2 to the Micro Column. Centrifuge for 15s at 12000 rpm at room temperature. Discard the flow-through.
13. Add 500 µl TWB2 to the Micro Column. Centrifuge for 3 min at 14000 rpm at room temperature. Discard the collection tube with the flow-through.

14. Place the Micro Column in a new 1.5 ml microtube. Add 30µl RNase-free water directly to the Micro Column membrane and incubation in RT for 3-5min then Centrifuge for 75s at 13000 rpm to elute the RNA.

    Note: If the expected RNA yield is more than the yield from the previous step, put the Micro Column on a new microtube and add another 30µl RNase-free water. Centrifuge for 1 min at 13000 rpm. However, it is possible to pass the flow-through from step 13 once more to obtain RNA with a higher concentration.