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)

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Kit Content

Component	100 preps
RLB	60ml
TWB1 (concentrate)	2 x 16ml
TWB2 (concentrate)	2 x 15ml
Nuclease-free RBC Lysis buffer Just included in RNJia Phenol-free PB Kit, (With RBC Lysis Buffer)	2×375ml
Nuclease-free Water	10ml
HiPure DR Column	100
Collection Tube	100

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 2×10^6	350
0.5 to 1.5	2×10^6 to 1×10^7	600

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

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 sample into HiPure DR Column, are carried out on ice.
- Before using the protocol for the first time, add 2-Mercaptoethanol to RLB, %2 of RLB volume. 2-Mercaptoethanol is commercially available and due to safety issue, is not provided in the kit. It can be order separately, by ROJETechnologies (Cat No BU983034) or Sigma-Aldrich (Cat No M3148).

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

- If RLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage condition and will not influence the efficiency of the buffer.
- 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 for the first time. Refer to washing buffer preparation section.

Process

- Collect 0.5 to 1.5ml blood into EDTA tubes. Add three volume of Nuclease-free RBC Lysis Buffer. Invert the tube 5 times and incubate at 4 ºC for 10min.
- Pulse vortex every 2min during incubation to intersperse the sample.
- Collect the WBCs by centrifugation at 2700 x g for 10 min at 4ºc.
- Discard the supernatant; add two volume of Nuclease-free RBC Lysis Buffer to the pellet, vortex until the pellet is dissolved completely.
- Collect the WBCs by centrifugation at 2700 g for 10min at 4ºC.
- 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 (see Table 1) and vortex or pipet to mix. After adding appropriate amount of RLB, use Micro pestle followed by homogenizer or syringe needle to homogenize the sample.

  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.

- Centrifuge the lysate for 3min at 15000 rpm. Carefully remove the supernatant by pipetting, and transfer it to a new RNase-free microcentrifuge tube.
- Add one volume of %100 ethanol to the homogenized lysate, and mix well by pulse vortexing for 15 s.

  Note: When purifying RNA from isolated cells, precipitates may be visible after addition of ethanol. This does not affect the procedure.
- Transfer up to 700µl of the sample, including any precipitate that may have formed, to HiPure DR Column placed in a 2ml collection tube (supplied in the kit box). Close the lid gently, and centrifuge for 1min at 13000 rpm. Discard the flow-through. Reuse the collection tube in step 10.
- Add 700µl TWB1 to the HiPure DR Column. Centrifuge for 1min at 13000 rpm at room temperature. Discard the flow-through.
- Add 500µl TWB2 to the HiPure DR Column. Centrifuge for 1min at 13000 rpm at room temperature. Discard the flow-through.
- Add 500µl TWB2 to the HiPure DR Column. Centrifuge for 3min at 14000 rpm at room temperature. Discard the collection tube with the flow-through.
- Place the HiPure DR Column in a new 1.5 ml microtube. Add 30-50µl RNase-free water directly to the HiPure DR Column membrane. Centrifuge for 1 min at 12000 rpm to elute the RNA.

  Note: If the expected RNA yield is more than the yield from pervious step, put the HiPure DR Column on a new microtube and add another 30-50µl RNase-free water. Centrifuge for 1min at 12000 rpm. However, it is possible to pass the flow-through from step 13 once more to obtain RNA with higher concentration.