## Recommended Starting Material

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

**Table.** Appropriate sample size and amount of RLB.

| Whole blood (ml) | WBCs Number | RLB Amount (µl) |
|---|---|---|
| Up to 0.5 | Up to $2 \times 10^6$ | 350 |
| 0.5 to 1.5 | $2 \times 10^6$ to $1 \times 10^7$ | 600 |

## Protocol

***Isolation of RNA (based on silica technology)***

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

### Some tips to know:

- All steps, before applying sample into spin column, are carried out on ice.
- Before using the protocol for the first time, add 2-Mercaptoethanol to RLB buffer, 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 buffer forms precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage condition and won't influence the efficiency of the buffer.

- Not forget to add the appropriate amount of Ethanol (96–100%) to TW1 and TW2 buffers (TWB1 and TWB2) as indicated on the bottle, before using for the first time.
- If working with RNA for the first time, read Appendix 1, in main handbook, carefully.

**Process**

1. Collect 0.5 to 1.5 ml blood into EDTA tubes. Add three volume of RBC Lysis Buffer (RNase-free). Invert the tube 5 times and incubate at 4ºc for 10 min.
2. Pulse vortex every 2 min during incubation to intersperse the sample.
3. Collect the WBCs by centrifugation at 2700 x g for 10min at 4ºc.
4. Discard the supernatant; add two volume of RBC Lysis Buffer (RNase-free) to the pellet, vortex until the pellet is dissolved completely.
5. Collect the WBCs by centrifugation at 2700g for 10 min at 4ºc.
6. Discard the supernatant. Disrupt the cells by adding RLB buffer (Supplemented with 2-βME). Loosen the cell pellet thoroughly by flicking the tube. Add the appropriate volume of RLB buffer (see Table) and vortex or pipet to mix. After adding appropriate amount of RLB buffer, 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.
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 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.

9. Transfer up to 700 µl of the sample, including any precipitate that may have formed, to spin column placed in a 2ml collection tube (supplied in the kit box). Close the lid gently, and centrifuge for 1min at 13000 rpm at room temperature (15-25℃). Discard the flow-through. Reuse the collection tube in step 10.
10. Add 700 µl TWB1 (TW1 buffer) to the spin column. Centrifuge for 1 min at 13000 rpm at room temperature (15-25℃). Discard the flow-through.
11. Add 500 µl TWB2 (TW2 buffer) to the spin column. Centrifuge for 1 min at 13000 rpm at room temperature (15-25℃). Discard the flow-through.
12. Add 500 µl TWB2 (TW2 buffer) to the spin column. Centrifuge for 3 min at 14000 rpm at room temperature (15-25℃). Discard the collection tube with the flow-through.
13. Place the spin column in a new 1.5 ml microtube. Add 30-50 µl RNase-free water directly to the spin column membrane. Centrifuge for 1 min at 12000 rpm at room temperature (15-25℃) to elute the RNA.

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