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 RNSol and chloroform amount are written in Table.

Table. Appropriate sample size and amount of RNSol, Chloroform and Ethanol amount

Whole blood	RNSol Amount	Chloroform Amount	Ethanol Amount
1 ml to 3 ml	500 µl	300 µl	400 µl
3 ml to 5 ml	700 µl	400 µl	400 µl
5 ml to 10 ml	1 ml	500 µl	400 µl

Protocol

Isolation of Total 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.
- 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.
- For frozen samples, thaw them to room temperature. Avoid repeated thawing and freezing of samples due to decreasing in RNA yield.
- If working with RNA for the first time, read Appendix 1, in main handbook, carefully.

Process

1. Collect 0.5 to 10 ml blood into EDTA tubes. Add 3 volume of RBC Lysis Buffer (RNase-free). Invert the tube 5 times and incubate at 4 ºc 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 2 volume of RBC Lysis Buffer (RNase-free) to the pellet, vortex until the pellet is dissolved completely.
5. Collect the WBCs by centrifugation at 2700 x g for 10 min at 4ºc.
6. Discard the supernatant. Add appropriate amount of RNSol Reagent to the sample (refer to the table).
7. Disrupt and homogenize the sample by selecting one of these ways:
    - After adding appropriate amount of RNSol, use Micropestle followed by homogenizer or syringe needle to homogenize the cell pellet.
    - After adding appropriate amount of RNSol, use TissueLyser or homogenizer to disrupt and homogenize the sample simultaneously.

    Optional: Homogenizing step is optional. It means that using homogenizer or syringe needle to homogenize the lysate can be omitted from the process.
8. After passing through the syringe for 5-10 times, pulse vortex for 1 min. incubate for 10 min at room temperature.

    Note: During isolating RNA from PBMC, it is necessary to thoroughly homogenize the sample and it is recommended to homogenize by passing the lysate 5-10 times through a blunt 20-gauge needle fitted to an RNase-free syringe.
9. Add appropriate amount of chloroform (refer to Table), vigorously shake it for 30 s. Then pulse vortex for 15 s and incubate at room temperature for 5 min.
10. Centrifuge at 4˚C for 12 min at 13000 rpm.

11. Transfer the aqueous phase (the upper phase) to a new tube. Be careful to avoid interfering the interphase.
12. Add an appropriate amount of Absolute Ethanol to the separated aqueous phase (refer to Table). Pulse vortex for 30 s.
13. Transfer the solution to a spin column placed in a 2 ml collection tube (supplied in the kit box). Centrifuge for 1 min at 13000 rpm at room temperature. Discard the flow-through.
14. Add 700 µl TWB1 (TW1 buffer) to the spin column. Centrifuge for 1 min at 13000 rpm at room temperature. Discard the flow-through.
15. Add 500 µl TWB2 (TW2 buffer) to the spin column. Centrifuge for 1 min at 13000 rpm at room temperature. Discard the flow-through.
16. Add 500 µl TWB2 (TW2 buffer) to the spin column. Centrifuge for 3 min at 14000 rpm at room temperature. Discard the collection tube with the flow-through.
17. Place the spin column in a new nuclease free 1.5 ml microtube. Add 30-100 µl RNase-free water directly to the spin 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 spin column on a new microtube and add another 30-100 µl RNase-free water. Centrifuge for 1 min at 12000 rpm. The yield will be nearly same as previous step. However, it is possible to pass the flow-through from step 17 once more to obtain RNA with higher concentration.