Total RNA Extraction Kit

Kit Contents:

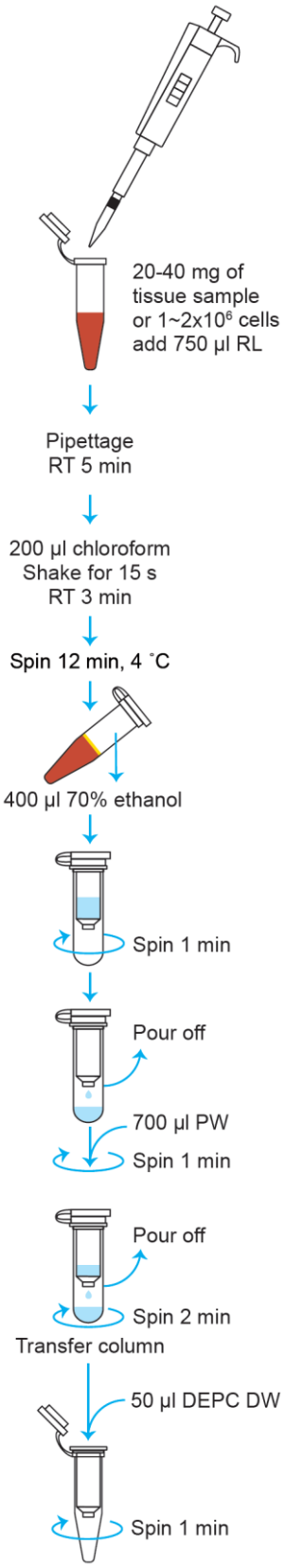
Components	50 reactions
RL Buffer (RNA Lysis Buffer)	2 x 20 ml
PW (Wash Buffer)	12 ml
DEPC-treated Water	3 ml
Spin Column	50 pcs
Collection Tube	50 pcs

Before Starting

1. Add 48 ml of absolute ethanol to the PW (only at the first use).

Reagent Not Provided

1. Chloroform
2. 70% ethanol



Protocol:

1. Cutting the tissue into the small pieces on a sterile petri dish by a scalpel to increase tissue lysis in the RL solution. Transfer 20-40 mg of tissue (20 mg for liver or spleen) or 150 µl blood or at least 1~2 x 10^6 cells (for cell cultures) into a 1.5 ml tube and add 750 µl of RL solution.
2. Pipetting the tissue into and out of the tip to avoid clumps. You can also homogenize hard tissue by homogenizer on ice. Incubate at room temperature for 5 min.
3. Add 200 µl of chloroform to the mixture. Shake it completely for 15 s and incubate for 3 min at room temperature.
4. Spin for 12 min at 13000 rpm at 4 ˚C.
5. Transfer 400 µl of the upper phase into a new 1.5 ml tube. Add 400 µl of 70% (96% ethanol for whole blood samples) ethanol to the mixture and mix them well.
6. Transfer mixture to the spin column. Do NOT touch upper rim of column. Spin for 1 min at 13000 rpm.
7. Pour off the flow-through of collection tube.
8. Add 700 µl of PW and spin for 1 min at 13000 rpm.
9. Pour off the flow-through of collection tube. (Optional: repeat step 8 and 9 with 500 µl of PW to have more pure RNA)
10. Spin for 2 min at 13,000 rpm to remove the remaining of the wash buffer. Transfer the spin column to a new 1.5 ml microtube.
11. Add 50 µl of DEPC-treated water, wait 3 min at room temperature or 57 ˚C (For more yield). If you want more concentration, add less DEPC-treated water (35 µl).
12. Spin for 1 min at 13,000 rpm to elute RNA from the column. Store RNA solution at -70 ˚C.