



## Total RNA Extraction Kit

for animal tissue, blood and cell culture

### Before Starting

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

### Reagents NOT Provided

1. Chloroform
2. 70% ethanol

### RNA Extraction 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  $\mu$ l blood or 1~2 x 10<sup>6</sup> cells (for cell cultures) into a 1.5 ml tube and add 750  $\mu$ l 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 150  $\mu$ l 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 13,000 rpm at 4 °C.
5. Transfer 400  $\mu$ l of the upper phase into a new 1.5 ml tube. Add 400  $\mu$ l of 70% 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 13,000 rpm.
7. Pour off the flow-through of collection tube.
8. Add 700  $\mu$ l of PW and spin for 1 min at 13,000 rpm.
9. Pour off the flow-through of collection tube. (Optional: repeat step 8 and 9 with 500  $\mu$ l 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  $\mu$ l of DEPC-treated water, wait 3 min at room temperature. If you want more concentration add less DEPC-treated water (30  $\mu$ l).
12. Spin for 1 min at 13,000 rpm to elute RNA from the column. Store RNA solution at -70 °C.

