E-zol RNA Reagent

*** Things to do before starting**

1. All centrifuge steps are done at **12,000 rpm at 4°C** in a microcentrifuge.

1. Add 1ml of E-zol RNA Reagent to **100mg tissue**
   (or precipitated blood RNA viruses from up to 10ml of blood or **10^6 cultured cells**).

2. Homogenize tissue samples in E-zol RNA Reagent using a glass-Teflon or polytron homogenizer (cultured cells can be lysed by repetitive pipetting: concentrated blood RNA viruses can be lysed by vigorous vortexing).

3. Leave the homogenates for **5 min at room temperature**.

4. Add **0.2ml of chloroform (not provided)** and mix vigorously.

5. Centrifuge at **12,000 rpm for 2 min at 4°C** to separate the phases,
   RNA is in the clear upper aqueous phase.

6. Transfer the RNA phase to a clean tube.

7. RNA is precipitated by adding **1 volume of isopropanol**, vortex,
   leave at **room temperature for 10 min**, and then centrifuge at **12,000 rpm for 15 min at 4°C**.

8. Remove the supernatant.

9. Wash the RNA pellet with **0.5ml ice cold 70% ethanol**, centrifuge at **12,000 rpm for 1 min at 4°C**, and carefully remove the supernatant.

10. A brief spin to make sure the RNA pellet is precipitated to the designated side wall of the tube and then carefully remove any residue supernatant without touching the RNA pellet.

11. Resuspend the RNA in a small volume of RNase-free water.

BENCH-TOP PROTOCOLS for E-zol RNA Reagent

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Please consult the E-zol RNA Reagent Handbook
before using these protocols for the first time.

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