Trizol method for RNA extraction

Aim of the experiment

The purpose of this experiment is to use Trizol to extract total RNA from E. coli BL21. Escherichia coli BL21 contains the target RNA induced by IPTG. TRIZOL is a new type of total RNA extraction reagent, which can directly extract total RNA from cells or tissues. It contains phenol, guanidine isothiocyanate and other substances, which can quickly disrupt cells and inhibit nucleases released by cells. TRIZOL can maintain the integrity of RNA when disrupting and lysing cells.

Materials

- Trizol
- Chloroform
- Isopropanol
- 75% Ethanol
- diethyl pyrocarbonate（DEPC）water

Procedure

1. Choose the microbial based on its OD value. Take 1 ml microbial whose OD value is between 0.5-0.6.

2. Centrifuge the EP tube for 3 minutes at 10000 rpm, and wash twice with sterile water.

3. Add 1ml Trizol and stir it.

4. Refrigerate it at -80℃.

5. Thawed the -80℃ sample and added 200ul chloroform.

6. Centrifuge the sample at 4℃, 12000 rpm for 15 minutes. Take 400ul or 500ul in 1.5ml EP tube. Add isopropyl alcohol to the same volume , stir it and let stand for 20 minutes

7. Centrifuge the sample at 4℃, 12000 rpm for 10 minutes, and abandon the supernatant.

8. Add 1ml 75% ethanol and centrifuge it at 1200 rpm for 10 minutes.

9. Suck 800ul supernatant and centrifuge it at 12000 rpm for 1 minute. Suck another 100ul supernatant and centrifuge it at 12000rpm for 1 minute. Blot it.

10. Open air drying on a clean bench.

11. Add 10ul DEPC and centrifuge it for 3 times. Measure the concentration and the purity of RNA and save it at -80℃.