TRIzol

Introduction

In order to allow the aptamer to put into effect in the environment, we need to extract the aptamer from E. coli by the means of TRIzol. This method would lyse the bacteria first, and than preciptate the nucleotide acid. all steps involving volatile or hazardous chemicals should be carried out in a chemical fume hood.

Reference:

Vibhute, M. A., Machatzke, C., Krümpel, S., Dirks, M., Bigler, K., Summerer, D., & Mutschler, H. (2026). Intracellular expression of a fluorogenic DNA aptamer using retron Eco2. *Elife*, *13*, RP99554.

Materials

- chloroform 200ul
- isoproponal 500ul
- sodium acetate 60ul
- ethanol 1ml
- TRI reagent 1ml
- MQ water 1ml

Procedure

Pre-culture

1. Culture 200ml BL21 in LB overnight
2. Distribute to two 50ml tube, 13200rpm 3 min 4ºC
3. Resuspend pellet with 1m/ TRI reagent, wait for 15 min
4. 13200rpm, 10 min, 4ºC
5. Transfer supernatant to new 1.5ml eppendorf
6. Add 200ul chloroform. shake for 15sec.
7. Centrifuge by 13200rpm, 15min, 4ºC
8. Transfer the aqueous phase to new 1.5ml ependoff
9. Add 60 ul sodium acetate(3M) and 500ul isoproponal

10. Incubate at -20º C for 2 hours.

11. Centrifuge by 13200rpm, 60min, 4ºC, and discard the supernatant

12. wash pellet with 1ml ethanol(70%)

13. Centrifuge by 13200rpm, 5min, 4ºC to wash the pellet.

14. Repeat step 12, 13 once.

15. Dry the sample for 10 min.

16. Resuspend pellet with 100ul MQ water.