

# Chloroform/Isopropanol purification of RNA

## Aim of the experiment

Due to the presence of DNA templates and impurities in the previous transcription process, we decided to purify RNA in order to carry out further experiments

## Materials

- Transcription system 20 $\mu$ L
- Chloroform 20 $\mu$ L (Quantity for one use only)
- Isopropanol 40 $\mu$ L
- 100% Ethanol 60 $\mu$ L
- 70% Ethanol 200 $\mu$ L
- DEPC water 150 $\mu$ L

## Procedure

1. 20 $\mu$ L transcription system: Centrifugation: 4°C, 12000rpm, 5min.
  2. Add equal volume of chloroform and oscillate for 15s. Let stand at room temperature for 15min.
  3. Centrifugation: 4°C, 12000rpm, 5min.
  4. Transfer the supernatant to the new 1.5ml EP tube. (Be careful not to poke the bottom layer)
  5. Add isopropanol of the same volume, mix well, and let stand for 20min.
  6. Centrifugation: 4°C, 12000rpm, 5min, remove supernatant.
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7. Add 3 times volume of 100% ethanol, swirl and oscillate, incubate at  $-80^{\circ}\text{C}$  for at least 1 h.
  8. Centrifugation:  $4^{\circ}\text{C}$ , 12000rpm, 1min, remove supernatant, be careful not to lose or puncture particles.
  9. Add 200 $\mu\text{l}$  70% ethanol (DEPC water preparation).
  10. Centrifugation:  $4^{\circ}\text{C}$ , 12000rpm, 5min, remove supernatant, pay attention not to lose or puncture particles.
  11. Use pipette to absorb excess liquid and dry the open air for 2-5min.
  12. After drying, add 10 $\mu\text{L}$  DEPC water, then centrifuge with finger flick, repeat 3 times.
  13. Measure the concentration.
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