

# RNA Extraction

## Aim of the experiment

This protocol is used to extract RNA from the Bacteria.

## Materials

- Roti-Aqua P/C/I (Roth, X985.1)
- Chloroform (Roth)
- Ice-cold 100% Ethanol (Roth)
- 70% Ethanol (Roth)
- 2M Sodium Acetate Stock Solution

## Procedure

1. Briefly centrifuge bacteria at 7000 rcf, discard supernatant and resuspend in 300 µl of H<sub>2</sub>O.
2. Directly add 300 µl of Phenol-Chloroform-Isoamylalcohol (25:24:1 ; pH 4.5-5) under the hood.
3. Mix thoroughly by pipetting up and down.
4. Incubate for 5 minutes - shake (careful!) in between.
5. Centrifuge for 5 minutes at 16,000 rcf.
6. Transfer the supernatant to a gel tube, add 300 µl Chloroform and shake well.
7. Centrifuge for 5 minutes at 16,000 rcf.
8. Transfer aqueous phase to new tube.
9. Add 10 µl 2M Sodium Acetate.
10. Add 400 µl of ice cold 100% ethanol, shake briefly.
11. Incubate at -80°C for minimal 1 hour (or longer).
12. Centrifuge at 4°C, 16 000 rcf for 15 minutes.
13. Discard supernatant, add 1 ml of 70% ethanol.
14. Centrifuge at 4°C, 16 000 rcf for 15 minutes.
15. Discard supernatant.
16. Spin in vacuum concentrator for 5 – 10 minutes (do not spin too long ; RNA-pellet is more sensitive than DNA).
17. Dissolve (invisible) pellet in 10-20 µl of nf H<sub>2</sub>O.