

RNA Extraction with DNase treatment

Aim of the experiment This protocol is used to extract RNA from the Bacteria and includes a subsequent DNA-digestion step to remove residual DNA contamination.

Materials

- Roti-Aqua P/C/I (Roth, X985.1)
- Chloroform (Roth)
- Ice-cold 100% Ethanol (Roth)
- 70% Ethanol (Roth)
- 2M Sodium Acetate Stock Solution
- NEB DNase I (RNase free) (New England Biolabs, M0303S)

Procedure

1. Briefly centrifuge bacteria at 7000 rcf, discard supernatant and freeze pellet in liquid N₂
2. Directly add 300 µl of Phenol-Chloroform-Isoamylalcohol (25:24:1; pH 4.5-5) and 300 µl of nuclease free H₂O 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 μ l 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 89 μ l of nf H_2O .
18. Add 10 μ l of 10X DNase I Reaction Buffer
19. Add 1 μ l of DNase I and incubate at 37°C for 15 Minutes
20. Add 100 μ l of Phenol-Chloroform-Isoamylalcohol (25:24:1;pH 4.5-5) under the hood.
21. Transfer the supernatant to a gel tube, add 100 μ l Chloroform and shake well.
22. Centrifuge for 5 minutes at 16,000 rcf.
23. Transfer aqueous phase to new tube.
24. Add 10 μ l 2M Sodium Acetate.
25. Add 400 μ l of ice cold 100% ethanol, shake briefly.
26. Incubate at -80°C for minimal 1 hour (or longer).
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