

Phenol-Chloroform precipitation

Aim of the experiment

This protocol is used to separate nucleic acids from solution, to obtain DNA/RNA for further analysis.

Materials

- PhaseGel tubes (braune Schachtel)
- Phenol-Chloroform-Isomethylethanol for DNA (Carl Roth)
- Chloroform
- 3M Natrium-Acetat
- 100% Ethanol at -80°C
- Nuclease free H₂O
- 1.5 ml microcentrifuge tubes

Procedure

1. Centrifuge the PhaseGel tube at 16,000 rcf for 5 minutes.
2. Add 100 µl sample.
3. Add equal volume of Phenol-Chloroform-Isomethylethanol for DNA.
4. Spin 5 minutes at 16,000 rcf at RT.
5. Repeat step 3 – 4 two times
6. add 100 µl Chloroform.
7. Spin 5 minutes at 16,000 rcf at RT.
8. Transfer supernatant (aqueous phase above gel) into a 1.5 ml sterile tube.
9. add 10 µl 3M Na-Acetat.
10. add 300 µl -80°C 100% Ethanol.
11. Vortex and incubate at -80°C for 1 hour.
12. Spin for 30 minutes at 16,000 rcf at 4°C.
13. Carefully pure out supernatant (do not lose DNA pellet).
14. Lyophilize the sample by using spinning-concentrator at 45°C for approximately 10 minutes.
15. Elute the sample in 25 µl nf H₂O.
16. Check the DNA concentration with NanoDrop, dilute if necessary.
17. Store DNA at -20°C.