Innuscreen
innovative Sensor Technology

innuPREP DNA Mini Kit 2.0

Extraction of DNA from blood samples

Before starting:

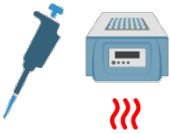
Add indicated amount of dd H_2O to lyophilized Proteinase K

Prepare Washing Solution BS according to the instruction

Starting material

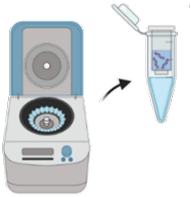
1. Transfer 200 (400) µL blood sample into a reaction tube

Lysis and Binding



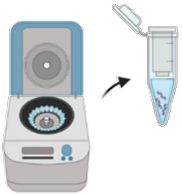
2. Add 200 (400) µL Lysis Solution SLS + 20 (30) µL Proteinase K
3. Vortex, incubate sample 10 min at 60°C
4. Optional: Add 1-2 µL RNase (10 mg/mL)
5. Add 350 (700) µL Binding Solution BL, mix by pipetting up and down

Binding and Washing



6. Apply 750 µL (and then residual) sample onto spin filter
7. Centrifuge 1 min at 11.000 x g, discard the filtrate
8. Add 400 µL Washing Solution C
9. Centrifuge 1 min at 11.000 x g, discard the filtrate
10. Add 600 µL Washing Solution BS
11. Centrifuge 1 min at 11.000 x g, discard the filtrate
12. Add 600 µL Washing Solution BS
13. Centrifuge 1 min at 11.000 x g, discard the filtrate

EtOH Removal & Elution



14. Centrifuge 3 min at max. speed
15. Place spin filter into an Elution Tube
16. Add 200 µL RNase-free water or pre-heated (60°C) Elution Buffer, wait 2 min
17. Centrifuge 1 min at 11.000 x g, discard the spin filter
18. Store eluate at appropriate temperature

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Extraction of DNA from tissue samples or rodent tails

Before starting:

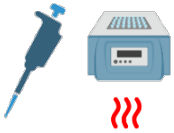
Add indicated amount of dd H_2O to lyophilized Proteinase K

Prepare Washing Solution MS according to the instruction

Starting material

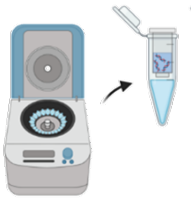
1. Weigh up to 20 mg tissue sample/pieces of rodent tail into a reaction tube

Lysis and Binding



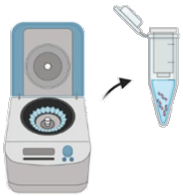
2. Add 400 µL Lysis Solution CBV + 30 µL Proteinase K
3. Incubate up to 3h at 56°C until sample is completely lysed
4. Transfer lysed sample into new reaction tube
5. Optional: Add 1-2 µL RNase A (10 mg/mL)
6. Add 200 µL Binding Solution SBS, mix by pipetting up and down

Washing



7. Apply sample onto spin filter
8. Centrifuge 2 min at 11.000 x g, discard the filtrate
9. Add 650 µL Washing Solution MS
10. Centrifuge 1 min at 11.000 x g, discard the filtrate
11. Add 650 µL Washing Solution MS
12. Centrifuge 1 min at 11.000 x g, discard the filtrate
13. Add 300 µL Washing Solution MS

EtOH Removal & Elution



14. Centrifuge 3 min at 11.000 x g, discard the receiver tube
15. Place spin filter into an Elution Tube
16. Add 100-400 µL RNase free water or Elution Buffer, wait 2 min
17. Centrifuge 1 min at 11.000 x g, discard the spin filter
18. Store eluate at appropriate temperature

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Extraction of DNA from cell cultures

Before starting:

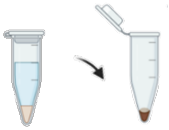
Add indicated amount of dd H_2O to lyophilized Proteinase K

Prepare Washing Solution MS according to the instruction

Starting material

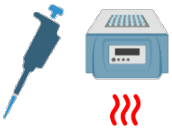
1. Transfer the liquid cell culture into a reaction tube

Pelleting



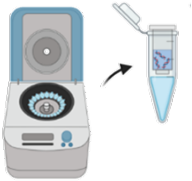
2. Centrifuge cells 10 min at 5.000 x g, discard the supernatant
3. Add 100 µL PBS Buffer, resuspend the pellet by pipetting up and down

Lysis and Binding



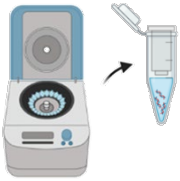
4. Add 300 µL Lysis Solution CBV + 25 µL Proteinase K, vortex
5. Incubate up to 30 min at 56°C until sample is lysed
6. Optional: Add 1-2 µL RNase A (10 mg/mL), incubate 5 min at RT
7. Add 200 µL Binding Solution SBS, vortex

Washing



8. Apply sample onto spin filter
9. Centrifuge 2 min at 11.000 x g, discard the filtrate
10. Add 650 µL Washing Solution MS
11. Centrifuge 1 min at 11.000 x g, discard the filtrate
12. Add 650 µL Washing Solution MS
13. Centrifuge 1 min at 11.000 x g, discard the filtrate
14. Add 300 µL Washing Solution MS
15. Centrifuge 3 min at max. speed, discard the receiver tube
16. Place spin filter into an Elution Tube

EtOH Removal & Elution



17. Add 100-400 µL RNase free water or Elution Buffer, wait 2 min
18. Centrifuge 1 min at 11.000 x g, discard the spin filter
19. Store eluate at appropriate temperature

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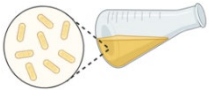
Extraction of DNA from bacterial cell cultures

Before starting:

Add indicated amount of dd H_2O to lyophilized Proteinase K

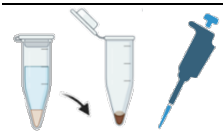
Prepare Washing Solution MS according to the instruction

Starting material



1. Transfer bacterial culture into a reaction tube

Pelleting



2. Centrifuge adequately (e.g. 10 min, 3.000 x g), discard supernatant
3. Add 100 µL TE-Buffer, resuspend the pellet by pipetting up and down

Optional Pre-Lysis (not included in the kit)

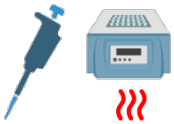
Gram (-): add 20 µL Lysozyme (10mg/mL), incubate 30 min at 37°C

Gram (+): add 20 µL Lysozyme + 5 µL Mutanolysin (0.4 U/µL), incubate 30 min at 37°C

Staphylococcus: add 10 µL Lysostaphin (0.4 U/µL), incubate 30 min at 37°C

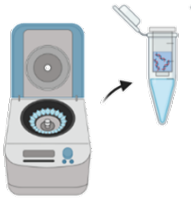
Alternatively: innuPREP Bacteria *Lysis Booster (IST) according manual*

Lysis and Binding



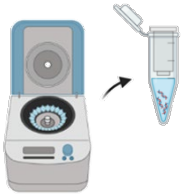
4. Add 280 µL Lysis Solution CBV + 20 µL Proteinase K, vortex
5. Incubate 30 min at 60°C (550 rpm)
6. Optional: Add 2 µL RNase A (10 mg/mL), incubate 10 min at RT
7. Add 200 µL Binding Solution SBS, mix by pipetting up and down

Washing



8. Apply sample onto spin filter
9. Centrifuge 2 min at 11.000 x g, discard the filtrate
10. Add 650 µL Washing Solution MS
11. Centrifuge 1 min at 11.000 x g, discard the filtrate
12. Repeat step 10 and 11
13. Add 300 µL Washing Solution MS

EtOH Removal & Elution



14. Centrifuge 3 min at max. speed, discard the receiver tube
15. Place spin filter into an Elution Tube
16. Add 50-200 µL RNase free water or Elution Buffer, wait 2 min
17. Centrifuge 1 min at 11.000 x g, discard the spin filter
18. Store eluate at appropriate temperature

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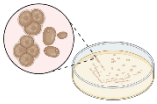
Extraction of DNA from yeast cell cultures

Before starting:

Add indicated amount of dd H_2O to lyophilized Proteinase K

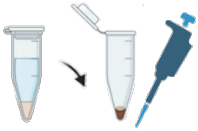
Prepare Washing Solution MS according to the instruction

Starting material



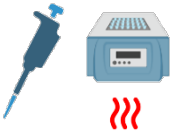
1. Transfer yeast culture to a tube

Pelleting



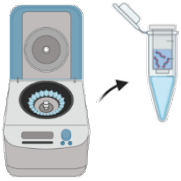
2. Centrifuge cells 10 min at 5.000 x g, discard the supernatant
3. Add 120 µL Yeast Digest Buffer, resuspend by pipetting up and down
4. Add 10 µL Lyticase (not included in the kit), incubate 30 min at 37°C

Lysis and Binding



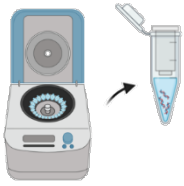
5. Add 280 µL Lysis Solution CBV + 20 µL Proteinase K, vortex
6. Incubate sample 30 min at 60°C (550 rpm)
7. Optional: Add 1-2 µL RNase A (10mg/mL), incubate 5 min at RT
8. Add 200 µL Binding Solution SBS, vortex

Washing



9. Apply sample onto spin filter
10. Centrifuge 2 min at 11.000 x g, discard the filtrate
11. Add 650 µL Washing Solution MS
12. Centrifuge 1 min at 11.000 x g, discard the filtrate
13. Add 650 µL Washing Solution MS
14. Centrifuge 1 min at 11.000 x g, discard the filtrate
15. Add 300 µL Washing Solution MS

EtOH Removal & Elution



16. Centrifuge 3 min at max. speed, discard the receiver tube
17. Place spin filter into an Elution Tube
18. Add 100-400 µL RNase free water or Elution Buffer, wait 2 min
19. Centrifuge 1 min at 11.000 x g, discard the spin filter
20. Store eluate at appropriate temperature

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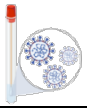
Extraction of DNA from buccal swabs

Before starting:

Add indicated amount of dd H_2O to lyophilized Proteinase K

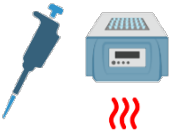
Prepare Washing Solution MS according to the instruction

Starting material



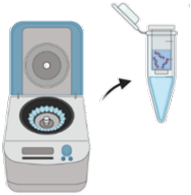
1. Place swab into a reaction tube
   (leave the swab in the tube during complete lysis)

Lysis and Binding



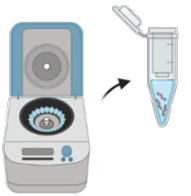
2. Add 400 µL Lysis Solution CBV + 25 µL Proteinase K, vortex
3. Incubate 10-15 min at 50°C
4. Remove the swab and squeeze it on the tube wall
5. Optional: Add 2 µL RNase A (10mg/mL), incubate 10 min at RT
6. Add 200 µL Binding Solution BL, mix by pipetting up and down

Washing



7. Apply sample onto spin filter
8. Centrifuge 2 min at 11.000 x g, discard the filtrate
9. Add 650 µL Washing Solution MS
10. Centrifuge 1 min at 11.000 x g, discard the filtrate
11. Add 650 µL Washing Solution MS
12. Centrifuge 1 min at 11.000 x g, discard the filtrate
13. Add 300 µL Washing Solution MS

EtOH Removal & Elution



14. Centrifuge 3 min at max. speed, discard the receiver tube
15. Place spin filter into an Elution Tube
16. Add 100-400 µL RNase free water or Elution Buffer, wait 2 min
17. Centrifuge 1 min at 11.000 x g, discard the spin filter
18. Store eluate at appropriate temperature