

innuPREP RNA Mini Kit 2.0

Extraction of RNA from up to 20 mg tissue sample

Before starting: Prepare Washing Solution HS and LS according to the instruction

Starting
material



1. Weigh up to 20 mg fresh or frozen tissue sample into a reaction tube

Homogenization
and Lysis



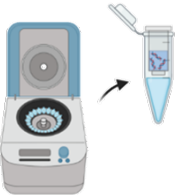
2. Add 450 μ L Lysis Solution RL to the sample
3. Homogenize (bead-based/ rotor-stator homogenizer, liquid nitrogen)

Binding



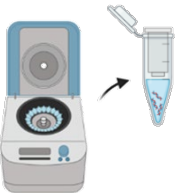
4. Centrifuge 1 min at max. speed to spin down unlysed material
5. Transfer supernatant onto Spin Filter D
6. Centrifuge 2 min at 11.000 x g, discard the spin filter (keep filtrate)
7. Add ~ 400 μ L 70% EtOH to the filtrate, mix

Washing



8. Transfer onto Spin Filter R
9. Centrifuge 2 min at 11.000 x g, discard the filtrate
10. Add 500 μ L Washing Solution HS
11. Centrifuge 1 min at 11.000 x g, discard the filtrate
12. Add 700 μ L Washing Solution LS
13. Centrifuge 1 min at 11.000 x g, discard the filtrate
14. Optional: Additional washing step with 700 μ L 80% EtOH

EtOH Removal &
Elution



15. Centrifuge 2 min at 11.000 x g, place spin filter into an Elution Tube
16. Add 30-80 μ L RNase-free water, wait 1 min
17. Centrifuge 1 min at 11.000 x g
18. Store eluate at appropriate temperature

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Extraction of RNA from up to 5×10^6 eukaryotic cells

Before starting: Prepare Washing Solution HS and LS according to the instruction

Lysis



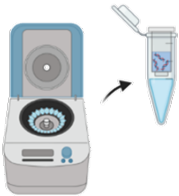
1. Add 400 μ L Lysis Solution RL to the cell pellet, incubate for 2 min
2. Resuspend by pipetting up and down, incubate for 3 min

Binding



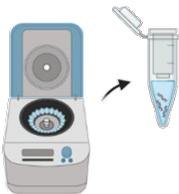
3. Transfer lysate onto Spin Filter D
4. Centrifuge 2 min at 11.000 x g, discard spin filter (keep filtrate)
5. Add ~ 400 μ L 70% EtOH to the filtrate, mix

Washing



6. Transfer onto Spin Filter R
7. Centrifuge 2 min at 11.000 x g, discard the filtrate
8. Add 500 μ L Washing Solution HS
9. Centrifuge 1 min at 11.000 x g, discard the filtrate
10. Add 700 μ L Washing Solution LS
11. Centrifuge 1 min at 11.000 x g, discard the filtrate
12. Optional: Additional washing step with 700 μ L 80% EtOH

EtOH removal & Elution



13. Centrifuge 3 min at 11.000 x g, place spin filter into an Elution Tube
14. Add 30-80 μ L RNase-free water, wait 1 min
15. Centrifuge 1 min at 11.000 x g
16. Store eluate at appropriate temperature

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Extraction of RNA from up to 5×10^9 bacterial cells

Before starting: Prepare Washing Solution HS and LS according to the instruction

Starting material



1. Transfer culture into 2 mL tube

Pelleting



2. Centrifuge adequately (e.g. 10 min, 3.000 x g), discard supernatant
3. Add 200 μ L TE-Buffer, resuspend the pellet by pipetting up and down

Optional Pre-Lysis

(not included in the kit)

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

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

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

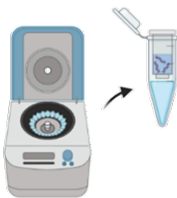
Alternatively: innuPREP Bacteria Lysis Booster (IST) according to manual

Lysis & Binding



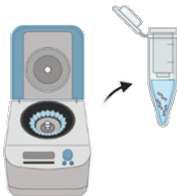
4. Add 450 μ L Lysis Solution RL to the pellet, mix, incubate for 3 min
5. Transfer supernatant onto Spin Filter D
6. Centrifuge 2 min at 11.000 x g, discard the spin filter (keep filtrate)
7. Add ~ 600 μ L 70% EtOH to the filtrate, mix

Washing



8. Transfer onto Spin Filter R
9. Centrifuge 1 min at 11.000 x g, discard the filtrate (repeat with rest)
10. Add 500 μ L Washing Solution HS
11. Centrifuge 1 min at 11.000 x g, discard the filtrate
12. Add 700 μ L Washing Solution LS
13. Centrifuge 1 min at 11.000 x g, discard the filtrate
14. Optional: Additional washing step with 700 μ L 80% EtOH

EtOH Removals & Elution



15. Centrifuge 3 min at 11.000 x g, place spin filter into an Elution Tube
16. Add 30-80 μ L RNase-free water, wait 1 min
17. Centrifuge 1 min at 11.000 x g
18. Store eluate at appropriate temperature