version 25.04.22

PathoSense

2025

CONFIDENTIAL

Wet lab flow diagnostics PathoSense

WET LAB IV - DNA Clean Up

Viral and bacterial DNA clean up using CleanNA magnetics beads

Timing ~ 60 min / 12 samples

Reagents

- 70% Ethanol* (400 µL / sample)
- CleanNA magnetic beads (50 µL / sample, 1:1 ratio)
- DNase/RNase free water

*It is important to prepare fresh 70% ethanol each time, since ethanol attracts water when kept too long

Materials

- DNA-Lobind 1.5 mL Eppendorf tubes (3 / sample)
- Magnetic rack
- Wide-bore tips

- All surfaces should be cleaned with Hibiscrub, 70% Ethanol, and 10% Bleach in this order of cleaning. Prevent contamination.
- Aerosol-barrier tips should be used throughout the entire procedure.

- Perform all steps in the dedicated sample preparation area.

Protocol

1. **Label** three **1.5 mL Eppendorf tubes** for each sample
2. **Vortex** the CleanNA magnetic beads until all beads are suspended
3. Use **wide-bore tips** to add **50 µL CleanNA magnetic beads** to the first labeled eppendorf (1:1 ratio)
4. Add **50 µL sample** to each corresponding labeled eppendorf containing the magnetic beads
5. **Gently mix** the suspension by tapping the tube with your fingers and **quick spin**
6. **Incubate** the suspension for **5 minutes** at **room temperature**

   In the meanwhile prepare the fresh 70% ethanol:

   - It is important to prepare **fresh** 70% ethanol each time, since ethanol attracts water when kept too long
   - Add **3.64 ml 100% ethanol** (Molecular grade) and **1.56 ml DNase/RNase- free water** in a 15 mL Falcon tube.

7. Place the sample centered into the **magnet holder**, and incubate for **2 minutes**
8. **Remove the supernatant** using a P200 pipette tip, without disturbing the beads
9. Gently **wash the beads** using **200 µL fresh 70% ethanol**, without disturbing them
10. **Remove the ethanol**, and **repeat** the wash step (9)
11. **Remove the ethanol**, and spin the sample down
12. Place sample in **magnet holder** and **remove the residual of the ethanol** with P20 pipette tip
13. **Incubate** the tubes with open lids for **MAX 5 minutes at 50°C** to evaporate all the ethanol and to dry the beads

    Incubate until a '**dry**' pellet is observed and no more ethanol is visible

14. When a 'dry' pellet is observed **add 25 µL DNase/RNase-free** water straight on the pellet (DO NOT PIPET)
15. **Tap the tube** to dissolve the magnetic beads, quick spin
16. Incubate at **room temperature for 2 minutes** to elute the DNA

17. Incubate for **2 more minutes** on the **magnetic rack**

18. **Transfer the supernatant (25 µL)**to a new labeled eppendorf tube

19. Place the tube once more on **the magnetic rack for 2 minutes**

20. Again **transfer the supernatant (20 µL)** to the 3rd labeled eppendorf tube

21. The **concentration and quality of the DNA** in the sample can be checked with an appropriate assay such as Nanodrop or Quantifluor using 2 µL of the sample

Ideally, samples are processed immediately for library preparation. They can be stored at 4°C for up to 5 days.

Keep samples on ice and proceed with

WET LAB V - LIBRARY PREPARATION