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PathoSense

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CONFIDENTIAL

Wet lab flow diagnostics PathoSense

WET LAB II - Extraction

Viral and bacterial lysis and isolation of viral and bacterial DNA/RNA

Timing ~ 45 min / 6 samples

Reagents

- 2x DNA/RNA Shield, Zymo Research (200 µL)
- Viral DNA/RNA Buffer, Zymo Research* (800 µL)
- Viral RNA Wash Buffer, Zymo Research** (1000 µL)
- 95 - 100 % Ethanol (500 µL)
- DNase/RNase free water (35 µL)

*Before first use of a new bottle, add 0.5% (v/v) Beta-mercaptoethanol to the viral DNA/RNA buffer (125 µL/25mL)

**Before first use of a new bottle, add 96 mL 100% ethanol to Viral RNA Wash Buffer

Materials

- Benzonase-treated sample
- Zymo-Spin IIC-XL Column
- Collection tubes (6)
- DNA-Lobind 1.5 mL Eppendorf tube

- 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. Add **200 µL DNA/RNA Shield** to each sample and mix well by pipetting
2. Add **800 µL Viral DNA/RNA Buffer** to each sample and mix well by pipetting
3. Transfer 600 µL into a **Zymo-Spin IIC-XL Column** in a collection tube and centrifuge for 2 minutes at 16,000 xg
4. Transfer the column into a **new collection tube** and **reload with the leftover 600 µL** and centrifuge for 2 minutes at 16,000 xg
5. Transfer the column into a new collection tube and add 500 µL **Viral RNA Wash Buffer** to the column and centrifuge for 30 sec at 16,000 xg
6. Transfer the column into a new collection tube and add 500 µL **Viral RNA Wash Buffer** to the column and centrifuge for 30 sec at 16,000 xg
7. Transfer the column into a new collection tube and add 500 µL **95-100% ethanol** to the column and centrifuge for 1 minute at 16,000 xg
8. Transfer the column into a new collection tube and centrifuge for 1 minute at 20,000 xg (= **dry spin**)
9. Transfer the column into a **newly labeled 1.5 mL DNA Lobind tube** and add **35 µL DNase/RNase water** directly onto the centre of the column matrix, without touching the column with the tip and **incubate for 1 minute** at room temperature
10. Centrifuge* closed column in DNA-Lobind tube for **30 sec** at **16,000 xg**

*Place the cap of the tube in the opposite direction as the arrow, along with the movement of the centrifuge. This to avoid breaking off the cap of the tube. If this does happen, transfer the eluate to a new tube.

Keep eluted viral nucleic acids (RNA and DNA) on ice and proceed (preferentially) immediately with

WET LAB III - cDNA generation and PCR amplification

Safe stopping point

If the samples cannot be processed immediately, viral nucleic acids can be stored at -70°C for 1 month