Viral DNA and RNA Extraction from Faecal Specimens using QIAamp MinElute Virus Kits (Spin-Column Approach)

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Protocol status: Working
We use this protocol and it's working

DISCLAIMER

ABSTRACT

The QIAamp MinElute Virus Spin procedure comprises four steps (lyse, bind, wash, elute) and is carried out using QIAamp MinElute columns in a standard microcentrifuge. The QIAamp MinElute Virus Spin Kit can isolate viral RNA and DNA from a broad range of RNA and DNA viruses. The procedure suits plasma, serum, and other cell-free body fluids. Herein, the QIAamp MinElute Virus Kits were used to extract viral DNA and RNA from faecal specimens according to the manufacturer's instructions with minor modifications.

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1 Faecal specimens were vigorously vortexed and homogenised for 5 min.

2 The supernatants were collected after centrifugation at 15,000 x g for 10 min.

3 Approximately 200 µl of the supernatant from each sample was filtered through a 0.45 µm filter and 0.2 µm filter to remove eukaryotic cell and bacterium-sized particles.

4 The filtrates were then digested by DNase and RNase to digest unprotected nucleic acids.

5 25 µl of Protease or Proteinase K was pipetted into a 1.5 ml microcentrifuge tube, and then 200 µl of filtered supernatants were added into the microcentrifuge tube.

6 200 µl Buffer AL containing 28 µg/ml of carrier RNA was added to a tube and then incubated at 56°C for 15 minutes in a heating block or water bath.

7 Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid, and add 250 µl of ethanol (96–100%) to the sample, close the cap and mix thoroughly by pulse-vortexing for 15 s.

8 Incubate the lysate with the ethanol for 5 min at room temperature (15–25°C).

9 Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid. Carefully apply all of the lysate from the previous step onto the QIAamp MinElute column without wetting the rim.

10 Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube and discard the collection tube containing the filtrate.

11 Carefully open the QIAamp MinElute column, and add 500 µl of Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min.

12 Place the QIAamp MinElute column in a clean 2 ml collection tube and discard the collection tube containing the filtrate.

13 Carefully open the QIAamp MinElute column, and add 500 µl of Buffer AW2 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min.

14 Place the QIAamp MinElute column in a clean 2 ml collection tube and discard the tube containing the filtrate.

15 Carefully open the QIAamp MinElute column and add 500 µl of ethanol (96–100%) without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Discard the collection tube containing the filtrate.

16 Place the QIAamp MinElute column in a clean 2 ml collection tube. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min to dry the membrane completely.

17 Place the QIAamp MinElute column into a new 2 ml collection tube, open the lid, and incubate the assembly at 56°C for 3 min to dry the membrane completely.

18 Place the QIAamp MinElute column in a clean 1.5 ml microcentrifuge tube, and discard the collection tube with the filtrate.

19 Carefully open the lid of the QIAamp MinElute column and apply 20–150 µl of Buffer AVE or RNase-free water to the centre of the membrane.

20 Close the lid and incubate at room temperature for 1 min. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 1 min.

21 Extracted viral nucleic acids were stored at -80 °C for further analyses.