Quick-Start Protocol March 2016

QIAamp® MinElute® Media Kit

The QIAamp MinElute columns of the QIAamp MinElute Media Kit (cat. no. 57414) should be stored at 2–8°C upon arrival. Other kit components can be stored at room temperature (15–25°C) for up to 12 months if not otherwise stated on label.

Further information

- *QIAamp MinElute Media Handbook*: **www.qiagen.com/HB-0321**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- All centrifugation steps are carried out at room temperature (15–25°C).
- Equilibrate samples and Buffer AVE to room temperature.
- Dissolve any precipitates in Buffer ATL or Buffer AL by heating to 70°C.
- Prepare heating blocks, thermomixers or heated orbital incubators at 56°C and 70°C.
- Ensure that Buffer AW2 has been prepared and that carrier RNA dissolved in Buffer AVE and added to Buffer AL according to instructions in the handbook.

1. Pipet 80 µl of Buffer ATL into a 2 ml microcentrifuge tube (not provided).
2. Add 250 µl of sample into the 2 ml microcentrifuge tube.
3. Add 20 µl QIAGEN proteinase K. Close the lid and mix for 10 s.
4. Incubate at 56°C for 30 min. For optimal results, shake samples in a thermomixer at 900 rpm. If using a heating block, vortex the samples occasionally throughout the incubation period.
5. Briefly centrifuge the 2 ml tube to remove drops from the inside of the lid.
6. Add 250 µl of Buffer AL (containing 10 µg/ml of carrier RNA). Close the lid and mix by pulse-vortexing for 10 s.

7. Incubate at 70°C for 15 min with shaking. Centrifuge briefly.
8. Add 300 µl of ethanol (96–100%) to the sample. Close the lid and mix thoroughly by pulse-vortexing for 15 s. Incubate the lysate with the ethanol for 5 min at room temperature (15–25°C). Centrifuge briefly.
9. Insert a QIAamp MinElute column into a VacConnector on the vacuum manifold. Insert an extension tube into the open QIAamp MinElute column.
10. Carefully pipet all of the lysate from step 10 into the extension tube of the column. Switch on the vacuum pump. After the lysate has been completely drawn through, switch off the pump, and release the pressure to 0 mbar.
11. Apply 750 µl of Buffer AW2 into the extension tube of the column. Switch on the vacuum pump. After Buffer AW2 has been completely drawn through, switch off the pump, and release the pressure.
12. Apply 750 µl of ethanol (96–100%) into the extension tube of the column. Switch on the vacuum pump. After the ethanol has been completely drawn through, switch off the pump, and release the pressure.
13. Remove and discard the extension tube.
14. Close the lid of the column, place in the clean 2 ml collection tube saved from step 11 and centrifuge at full speed (20,000 x *g*, 14,000 rpm) for 3 min to dry the membrane completely. Discard the VacConnector.
15. Place the column in a clean 1.5 ml collection tube (provided) and discard the 2 ml collection tube. Open the lid of the column, and incubate the column at room temperature for 15 min.
16. Apply 120 µl of Buffer AVE to the center of the membrane. 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.

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