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Appendices

Appendix 1. QIAmp Viral RNA Mini Kit Protocol

1. Add 560 µL of prepared Buffer AVL containing carrier RNA into a clean 1.5 mL microcentrifuge tube. For sample volume larger than 140 µL, increase the amount of Buffer AVL proportionally and use a larger tube.
2. Add 140 µL of plasma, serum, urine, cell-culture supernatant, or cell-free body fluid to the tube. Mix by pulse-vortexing for 15 s.
3. Incubate at room temperature for 10 minutes, then briefly centrifuge the tube to remove drops from inside the lid.
4. Add 560 µL of 96-100% ethanol to the sample and mix by pulse-vortexing for 15 s. Briefly centrifuge the tube to remove drops from inside the lid. For sample volume larger than 140 µL, increase the amount of proportionally.
5. Apply 630 µL of the solution to the QIAmp Mini column in a 2 mL collection tube directly to the center. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 minute. Discard the flow-through and place the QIAmp Mini column into a clean 2 mL collection tube.
6. Carefully open the QIAmp Mini column and repeat step 5 until all the lysate has been loaded to the spin column
7. Carefully open the QIAmp Mini column and add 500 µL of Buffer AW1. Close the cap and centrifuge the tube at 6000 x g (8000 rpm) for 1 minute. Discard the flow-through and place the QIAmp Mini column into a clean 2 mL collection tube.
8. Carefully open the QIAmp Mini column and add 500 µL of Buffer AW2. Close the cap and centrifuge the tube at 20.000 x g (14.000 rpm) for 3 minutes. Discard the flow-through and place the QIAmp Mini column into a clean 1.5 mL microcentrifuge tube.
9. Add 60 µL of Buffer AVE to the column, close the cap and incubate at room temperature for 1 minute. Centrifuge at 6000 x g (8000 rpm) for 1 minute and store the elute at -20°C.