

**User-developed
protocol**

User-Developed Protocol:

Purification of viral RNA and DNA from 1000 µl of plasma, serum, and cell-free body fluids using the QIAamp® MinElute® Virus Vacuum Kit

This procedure has been adapted by customers and is for purification of viral RNA and DNA from plasma, serum, and cell-free body fluids using the QIAamp MinElute Virus Vacuum Kit. **The procedure has not been thoroughly tested and optimized by QIAGEN.**

IMPORTANT: Please read the *QIAamp MinElute Virus Vacuum Handbook*, paying careful attention to the “Safety Information” and “Important Notes” sections, before beginning this procedure.

Equipment and reagents to be supplied by the user

- Ethanol (96–100%)
- 5 ml tubes
- Pipet tips (pipet tips with aerosol barriers for preventing cross-contamination are recommended)
- Disposable gloves
- Heating block for lysis of samples at 56°C
- Microcentrifuge
- Vacuum manifold (e.g., QIAvac 24 Plus, cat. no. 19413)
- Vacuum pump capable of producing a vacuum of –800 to –900 mbar (e.g., QIAGEN Vacuum Pump)
- Optional: QIAvac Connecting System, cat. no. 19419, to connect the QIAvac 24 Plus to the QIAGEN vacuum pump. In combination with the QIAvac Connecting System, the QIAvac 24 Plus vacuum manifold can be used as a flow-through system. The sample flow-through, containing possible infectious material, is collected in a separate waste bottle.
- VacValves (cat. no. 19408), for regulating vacuum at each individual column if sample flow rates differ significantly
- VacConnectors (cat. no. 19407), for prevention of cross contamination caused by direct contact between QIAamp MinElute column and QIAvac 24 manifold
- Vacuum Regulator (QIAGEN cat. no. 19530; for easy monitoring of vacuum pressures and easy releasing of vacuum). The QIAvac Connecting System, cat. no. 19419, has an integrated vacuum regulator

Addition of carrier RNA to Buffer AL*

Add 310 µl Buffer AVE to the tube containing 310 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at -20°C. Do not freeze-thaw the aliquots of carrier RNA more than three times.

Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AVE and then added to Buffer AL. Gently mix by inverting the tube 10 times. To avoid foaming, do not vortex.

Calculate the volume of Buffer AL/carrier RNA-mix needed per batch of samples by selecting the number of samples to be simultaneously processed from Table 1.

Table 1. Volumes of Buffer AL and Carrier RNA/Buffer AVE-Mix Required for the QIAamp MinElute Virus Vacuum Procedure.

No. samples	Vol. Buffer AL (ml)	Vol. carrier RNA/AVE (µl)	No. samples	Vol. Buffer AL (ml)	Vol. carrier RNA/AVE (µl)
1	1.1	6.2	13	14.3	80.01
2	2.2	12.3	14	15.4	86.
3	3.3	18.5	15	16.5	92.4
4	4.4	24.6	16	17.6	98.6
5	5.5	30.8	17	18.7	104.7
6	6.6	37.0	18	19.8	110.9
7	7.7	43.1	19	20.9	117.0
8	8.8	49.3	20	22.0	123.2
9	9.9	55.0	21	23.1	129.4
10	11.0	61.6	22	24.2	135.5
11	12.1	67.8	23	25.3	141.7
12	13.2	73.9	24	26.4	147.8

Note: The sample-preparation procedure is optimized for 5.6 µg of carrier RNA per sample. If less carrier RNA has been shown to be better for your amplification system, transfer only the required amount of dissolved carrier RNA to the tubes containing Buffer AL. For each microgram of carrier RNA required per preparation, add 2 µl dissolved carrier RNA per milliliter of Buffer AL. (Use of less than 5.6 µg carrier RNA per sample must be validated for each particular sample type and downstream assay.)

* Contains chaotropic salt, which is an irritant. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach.

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- Equilibrate Buffer AVE to room temperature for elution in step 15.
- Prepare a 56°C heating block for use in step 4 and 14.
- Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to instructions on pages 14–16 of the *QIAamp MinElute Virus Vacuum Handbook*, November 2002.
- Add carrier RNA reconstituted in AVE to Buffer AL according to instructions listed in table 1.
- All centrifugation steps are carried out at room temperature.
- For processing using VacConnectors and VacValves, set up the QIAvac 24 as described in the *QIAamp MinElute Virus Vacuum Handbook*, November 2002.

Procedure

1. **Pipet 150 µl QIAGEN Protease into a 5 ml tube (not provided).**
2. **Add 1000 µl of plasma or serum into the 5 ml tube.**
3. **Add 1000 µl of Buffer AL (containing 11.2 µg/ml of carrier RNA), close the cap and mix by pulse-vortexing for 15 s.**

In order to ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution.

Note: Do not add QIAGEN Protease directly to Buffer AL.

4. **Incubate at 56°C for 15 min.**
5. **Briefly centrifuge the 5 ml tube to remove drops from the inside of the lid.**
6. **Add 1200 µl of ethanol (96–100%) to the sample, close the cap and mix thoroughly by pulse-vortexing for 15 s. Incubate the lysate with the ethanol for 10 min at room temperature (15–25°C).**

Note: If the ambient temperature exceeds 25°C, ethanol should be cooled on ice before adding to the lysate.

7. **Briefly centrifuge the 5 ml tube to remove drops from the inside of the lid.**
8. **Insert the QIAamp MinElute column into the VacConnector on the vacuum manifold. Insert an extension tube into the open QIAamp MinElute column.**

Note: Keep the collection tube for the dry spin in step 13.

9. **Carefully apply all of the lysate from step 7 into the extension tube of the QIAamp MinElute column. Switch on the vacuum pump. After all lysates have been drawn through the QIAamp MinElute column, switch off the vacuum pump and release the pressure to 0 mbar.**

If the lysates from individual samples have not completely passed through the membrane despite the VacValves of all other MinElute Columns being closed, place the QIAamp MinElute column into a clean 2 ml collection tube (not provided), close the cap, and centrifuge at full speed for 3 min or until it has completely passed through. Additional collection tubes can be purchased separately, see "Ordering Information" in the *QIAamp MinElute Virus Vacuum Handbook*.

Note: For fast and convenient release of the vacuum pressure the Vacuum Regulator should be used, see "Ordering Information" in the *QIAamp MinElute Virus Vacuum Handbook*.

10. **Apply 600 µl of Buffer AW1 to the QIAamp MinElute column. Remove and discard the extension tube before the vacuum is switched on. After all of Buffer AW1 has been drawn through the QIAamp MinElute column, switch off the vacuum pump and release the pressure to 0 mbar.**

Note: To avoid cross contaminations be careful to not take tubes across neighboring QIAamp MinElute columns extension tube removal.

11. **Apply 750 µl of Buffer AW2 to the QIAamp MinElute column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer AW2 has been drawn through the QIAamp MinElute column, switch off the vacuum pump and release the pressure to 0 mbar.**
12. **Apply 750 µl of ethanol (96–100%) to the QIAamp MinElute column. Leave the lid of the column open, and switch on the vacuum pump. After all of ethanol has been drawn through the column, switch off the vacuum pump and release the pressure to 0 mbar.**
13. **Close the lid of the QIAamp MinElute column, remove it from the vacuum manifold, and discard the VacConnector. Place the QIAamp MinElute column in a clean 2 ml collection tube saved from step 8, and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.**
14. **Place the QIAamp MinElute Column into a new 2 ml collection tube (not provided), open the lid, and incubate the assembly at 56°C for 3 min to dry the membrane completely.**
15. **Place the QIAamp MinElute column in a clean 1.5 ml microcentrifuge tube (provided) and discard the collection tube with the filtrate. Carefully open the lid of the QIAamp MinElute column, and apply 20–150 µl of Buffer AVE, or RNase-free water 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.**

IMPORTANT: Ensure that the elution buffer is equilibrated to room temperature. If elution is done in small volumes (<50 µl) the elution buffer must be dispensed onto the center of the membrane for complete elution of bound RNA and DNA.

Elution volume is flexible and can be adapted according to the requirements of the downstream applications. Remember that the recovered eluate volume will differ by approximately 5 µl from the elution volume applied onto the column.

**User-developed
protocol**

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature/handbooks/default.aspx. Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp.

Trademarks: QIAGEN[®], QIAamp[®], MinElute[®] (QIAGEN Group).
QA32 © 2004–2010 QIAGEN, all rights reserved.