

QIAamp® 96 Viral RNA Kit

The QIAamp 96 Viral RNA Kit (cat. no. 52962) should be stored at room temperature (15–25°C). Under this condition, the components are stable for 12 months without showing any reduction in performance and quality, unless otherwise indicated on the label. This protocol can be executed using various S-Blocks and 96-well elution plates. A list of compatible items can be found at the QIAamp 96 Viral RNA product page.

We recommend using a Sigma® 4-16S centrifuge (Sigma Laborzentrifugen GmbH) and a 2 x 96 plate rotor to centrifuge QIAamp 96 plates at 6000 rpm (5788 x *g*). If an alternative centrifuge with lower *g*-force is used, centrifugation times need to be adapted respectively. Centrifugation times for processing with a lower *g*-force than 5788 x *g*, but with at least 3486 x *g*, are given in the protocol in brackets.

Further information

- *QIAamp 96 Viral RNA Handbook*: www.qiagen.com/HB-2814
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: support.qiagen.com

Notes before starting

- Dissolve lyophilized Carrier RNA in 1550 µl Buffer AVE to a final concentration of 1 µg/µl.
- Add 10 µl of dissolved Carrier RNA solution (1 µg/µl) per milliliter of Buffer AVL. Gently mix by inverting the tube 10 times. To avoid foaming, do not vortex. Buffer AVL–carrier RNA should be prepared fresh and is stable at 2–8°C for up to 48 hours.
- Equilibrate all buffers to room temperature before starting.
- Perform QIAamp 96 plate centrifugation at least at $\geq 3486 \times g$. If centrifugation speed is $\leq 5788 \times g$, please prolong centrifugation time as stated in the brackets. Do not perform cooled centrifugation, because that could interfere with the evaporation of the ethanol.

Procedure

1. Pipet 560 µl Buffer AVL–carrier RNA into each well of an S-Block.
2. Add 140 µl sample and mix thoroughly by pipetting up and down 8 times.
3. Seal the S-Block with Tape Pad and incubate at room temperature for 10 min.

4. Carefully remove the Tape Pad and add 560 μl ethanol (96–100%). Mix thoroughly by pipetting up and down 8 times.
5. Place a QIAamp 96 Plate on a new S-Block. Pipet 630 μl of the solution from step 4 into the QIAamp 96 Plate. Seal the QIAamp 96 Plate with an AirPore tape sheet.
6. Load the QIAamp 96 plate and S-block assembly into a rotor bucket. Centrifuge for 4 min at 5788 $\times g$ (7min with at least 3486 $\times g$). If liquid has not completely passed through the membrane, centrifuge again.
7. Carefully remove the AirPore tape sheet and repeat steps 5 and 6 with the remaining lysate. Discard the S-Block that contains the flow-through.
8. Place the QIAamp 96 Plate on a new S-Block. Remove the AirPore tape sheet and add 500 μl Buffer AW1 to each well. Seal the QIAamp 96 Plate with a new AirPore tape sheet. Load the S-Block and the QIAamp 96 Plate with the carrier into a rotor bucket. Centrifuge for 4 min at 5788 $\times g$ (7min with at least 3486 $\times g$).
9. Remove the AirPore tape sheet and add 500 μl Buffer AW2. Seal the QIAamp 96 Plate with new AirPore tape sheet. Load the S-Block and the QIAamp 96 Plate with the carrier into a rotor bucket. Centrifuge for 5 min at 5788 $\times g$ (7min with at least 3486 $\times g$).
10. Remove the AirPore tape sheet and add 250 μl 96% EtOH. Seal the QIAamp 96 Plate with a new AirPore tape sheet. Load the S-Block and the QIAamp 96 Plate with the carrier into a rotor bucket. Centrifuge for 5 min at 5788 $\times g$ (7min with at least 3486 $\times g$). Discard the S-Block with the flow-through.
11. Place the QIAamp 96 well plate on a new S-Block. Remove the AirPore tape sheet and load the S-Block and QIAamp 96 Plate with the carrier into a rotor bucket. Centrifuge for 10 min at 5788 $\times g$ (25 min with at least 3486 $\times g$) without AirPore tape sheet to dry the membrane. Discard the S-Block.
12. Place the QIAamp 96 Plate in a 96-well elution plate. Add 80 μl Buffer AVE and incubate at room temperature for 1 min.
13. Load the 96-well elution plate and QIAamp 96 Plate with the carrier into a rotor bucket. Centrifuge for 4 min at 5788 $\times g$ (7min with at least 3486 $\times g$). Seal the elution plate for storage.

Document Revision History

Date	Changes
09/2020	Updated "Notes before starting" section. Revised several procedure steps.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.

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