

High Pure 16 System Viral Nucleic Acid Kit

for up to 6 x 16 isolations of viral nucleic acids for PCR or RT-PCR

Cat. No. 12 011 816 001

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Principle

The High Pure 16 System Viral Nucleic Acid Kit is designed for the simultaneous purification of multiple viral templates from serum or plasma. Nucleic acids can be applied in PCR or RT-PCR directly after elution in nuclease-free water. The special design of the 16 System devices allows 16 samples (or multiples thereof) to be processed in parallel. The use of the 16 system rack set simplifies handling of higher sample numbers by minimizing the risk of cross contamination.

The samples are lysed by incubation with a special buffer containing Proteinase K and guanidine hydrochloride that releases nucleic acids. A highly efficient reaction is obtained at elevated temperatures. Subsequently, the liquid is centrifuged through a glass fiber filter. During this process, the nucleic acids are bound specifically to the surface of the glass fibers. Unbound substances are removed by centrifugation. The adsorbed nucleic acids are washed and eluted with an aqueous solution.

Starting material

Research samples may be 200 µl of:

- Serum
- K₃-EDTA or sodium-citrate plasma
- Cell culture supernatant

! *Samples containing precipitates must be centrifuged before purification.*

Application

- Preparation of highly purified viral nucleic acids, which may be used directly as templates for standard or long-template PCR or RT-PCR.

Time required

- Total time: approx. 30 min for 32 samples
- Hands-on time: <10 min.

Benefits

- **Simultaneous and time-saving handling of large samples numbers**, special racks allow a parallel processing of 16 samples or multiples thereof (see Figure 24).
- **Reduces handling time by about 30%**, thus facilitating a high sample throughput.
- **Minimize** the risk of cross contamination
- **Track identity** of 16 samples at each isolation step

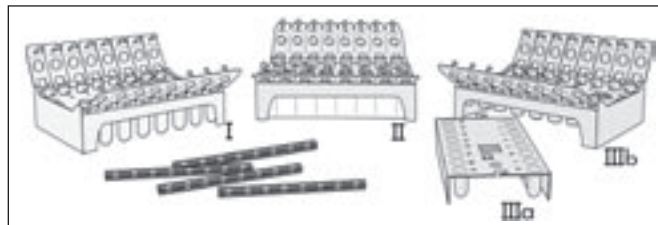
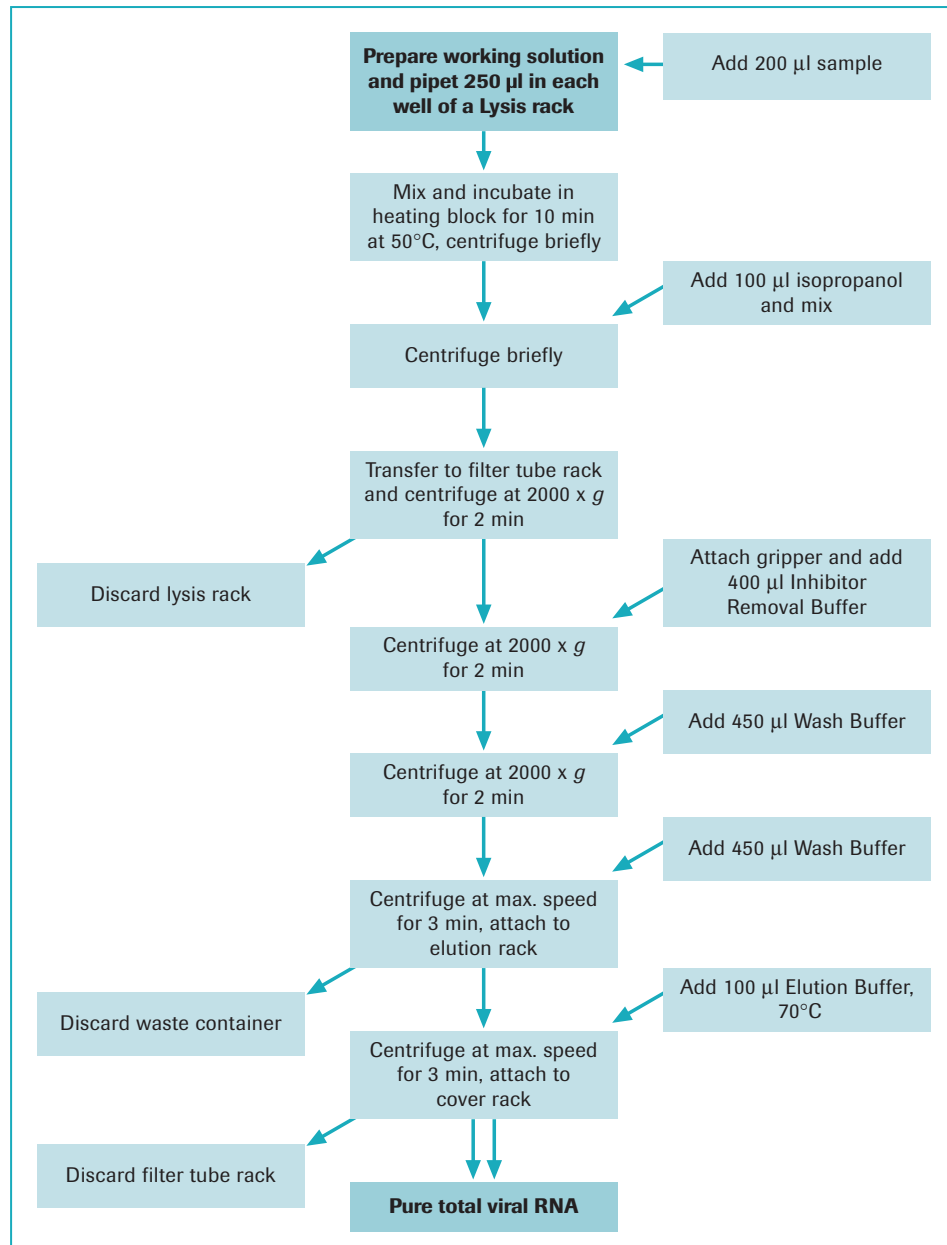


Figure 24: Special racks allow a parallel processing of 16 samples or multiples.

How to use the kit

I. Flow diagram



II. Kit contents

- Lysis/Binding Buffer (25 ml)
- Poly(A), lyophilizate (2 mg)
- Proteinase K, lyophilizate (100 mg)
- Inhibitor Removal Buffer (33 ml)
 - ⚠ Add 20 ml ethanol absolute before use
- Wash Buffer (20 ml)
 - ⚠ Add 80 ml ethanol absolute before use

- Elution Buffer (30 ml)
- High Pure 16 System Purification Rack Sets



The Lysis/Binding Buffer and the Inhibitor Removal Buffer contain guanidine-HCl which is an irritant. Wear gloves and follow usual safety precautions when handling.

III. Additional materials required

- Absolute ethanol
- Isopropanol
- Microtiter plate centrifuge capable of a 1800 x g centrifugal force and a minimum height of 35.6 mm for positioning of the High Pure 16 System racks
- General laboratory equipment

IV. Procedure for preparing nucleic acids from 200 µl samples of serum or plasma (research samples)



We recommend to compile 16 samples prior to start.

Before starting the purification reaction, prewarm an aliquot of the Elution Buffer to 70°C.

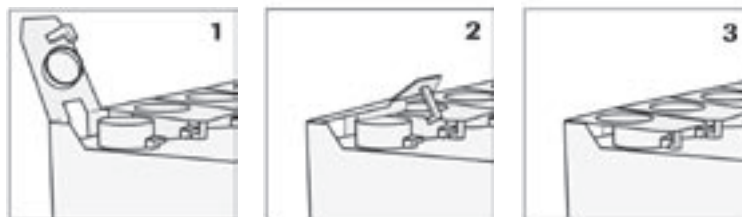


Figure 25: Lid positions.

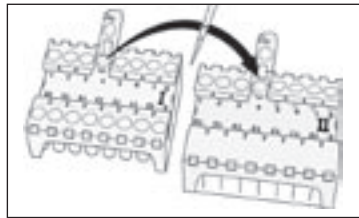
- 1 Take the lysis rack (I, transparent) and pipet 250 µl of freshly prepared working solution into the wells (e.g., using a multi-pipette). Push the lids down in covering position. The individual positions of the lids are shown in Figure 25: (1) open, (2) covering, (3) closed.
- 2 Add 200 µl serum or plasma to each well (split for >300 µl sample). After each pipetting step, push the lid further down until the snap mechanism engages to tightly close the well.
- 3 Immediately after the last pipetting step for 16 samples, thoroughly mix the samples for approx. 10 s by vortexing the lysis rack as shown below (Figure 26).



▶ Figure 26: Vortexing the Lysis Rack.

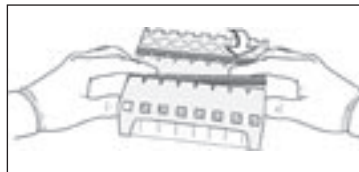


- 4 Incubate the lysis rack for 10 min at 50°C.
- 5 Briefly centrifuge the lysis rack.
- 6 Mix each sample with 100 µl isopropanol. Open only one lid at a time. Mix and centrifuge as described under step 3 and step 5 with all lids securely closed.
- 7 Take the Filter Tube Rack (II, yellow) including the waste container and push down all lids to cover the well openings. Transfer the lysates completely from the lysis rack(s) to the corresponding filter tube of the Filter Tube Rack. Open only one well at a time, respectively (Figure 27). Once a lysate transfer is completed, tightly close the corresponding wells. Discard the lysis rack.



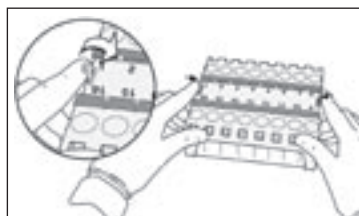
► **Figure 27: Transfer of lysates.**

- 8 Centrifuge at 2000 x *g* for 2 min in a Microtiter plate centrifuge. The material in the waste container absorbs the flow-through. It is not necessary to empty the waste container. This also applies to all corresponding washing steps.
- 9 Attach a gripper to simultaneously open 8 lids (Figure 28). Add 450 µl Wash Buffer (vial 4, blue cap) into each well (i.e. using a multipipette), and simultaneously close the lids. It is also possible to open/close lids individually.



► **Figure 28: Attach gripper.**

- 10 Repeat steps 8 and 9 once. For the final washing step increase centrifugation time to 3 min at maximum speed (e.g., 2200 x *g*).
- 11 To elute the nucleic acids remove the Filter Tube Rack from the waste container by pressing both snap links on the upper site of the Filter Tube Rack toward inside (Figure 29). Place the Filter Tube Rack onto the elution rack (IIIA, blue). Make sure both racks are properly positioned. Then slightly press down the Filter Tube Rack to connect both elements. Discard the waste container.



► **Figure 29: Removing Filter Tube Rack from the waste container.**

- 12 Add 100 µl of prewarmed Elution Buffer (vial 5, colorless cap) into each well and centrifuge at maximum speed for 3 min.
- 13 Remove the as Filter Tube Rack described under 11 and discard it. Place the cover rack (IIIB, blue) onto the elution rack, press down firmly, and bend the snap links slightly toward the outside. Close all lids.
- 14 The nucleic acids can be used directly in the PCR or RT-PCR.



- 15 If you want to go ahead, then use an aliquot of the eluted nucleic acids directly in PCR or RT-PCR.
- ▶ For RT-PCR use 3.5 – 6 µl of viral NA eluate.
 - ▶ For PCR use 10 – 25 µl of the viral NA eluate.
- If you want to stop, then store the eluted nucleic acids for later analysis.
- ▶ Viral DNA: at 2 to 8°C or –15 to –25°C
 - ▶ Viral RNA: at –80°C

V. Troubleshooting the High Pure protocols

- ▶ The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocol, see the General Troubleshooting Procedure for all High Pure kits on page 98 of this manual. For factors that may affect the High Pure 16 System Viral Nucleic Acid Kit, see page 107.

Typical results with the kit

Purification of M-MuLV Viral RNA



Figure 30: Detection of viral M-MuLV RNA in plasma by RT-PCR.

Sample volumes of 300 µl (lanes 3 & 4) and 200 µl (lanes 5 – 19) of plasma, spiked with dilution series of recombinant M-MuLV Viral Particles, were purified following the standard procedure of the High Pure 16 System Viral Nucleic Acid Kit.

The viral RNA was detected by RT-PCR using the Titan One Tube RT-PCR System generating a 228 bp DNA fragment (see Figure 30).

Lane 1: Roche Applied Science DNA MWM VI

Lane 2: Positive control: 5 ng M-MuLV RNA

Lane 3, 4: Samples with 2.5×10^6 pfu/ml, corresponding to 38 000 copies/PCR reaction

Lanes 5, 6: Samples with 2.5×10^6 pfu/ml, corresponding to 25 000 copies/PCR reaction

Lanes 7, 8: Samples with 5×10^5 pfu/ml, corresponding to 5 000 copies/PCR reaction

Lanes 9, 10: Samples with 2×10^5 pfu/ml, corresponding to 2 500 copies/PCR reaction

Lanes 11, 12: Samples with 5×10^4 pfu/ml, corresponding to 500 copies/PCR reaction

Lanes 13, 14: Samples with 2×10^4 pfu/ml, corresponding to 250 copies/PCR reaction

Lanes 15, 16: Samples with 5.2×10^3 pfu/ml, corresponding to 50 copies/PCR reaction

Lanes 17, 18: No viral particles

Lane 19: Negative control: RT-PCR with H₂O

Result: 50 copies of M-MuLV RNA could be detected with the kit

Purification of MS2 Phage RNA

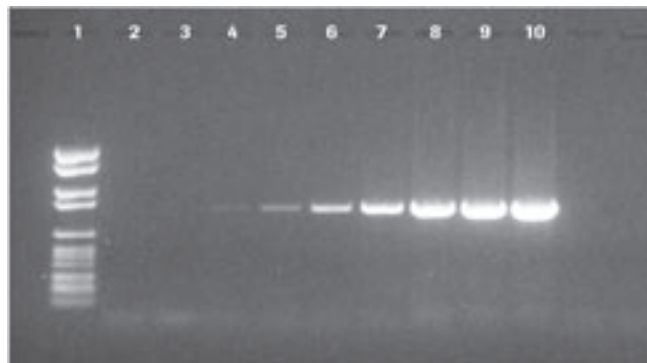


Figure 31: Detection of MS2 RNA by RT-PCR.

200 μ l of a dilution series of MS2 phage particles were applied to the High Pure 16 System Viral Nucleic Acid Kit and purified following standard procedures.

The purified MS2 RNA was analyzed via a standard two step RT-PCR procedure. After generation of a 3550 cDNA fragment, a 961 bp DNA product was amplified in the subsequent PCR step (see Figure 31).

Lane 1: Roche Applied Science DNA MWM VI

Lane 2: Negative control: RT-PCR with H₂O

Lane 3: No phage particles

Lane 4: Samples with 1×10^4 pfu/ml, corresponding to 35 copies/PCR reaction

Lane 5: Samples with 1×10^5 pfu/ml, corresponding to 350 copies/PCR reaction

Lane 6: Samples with 1×10^6 pfu/ml, corresponding to 3500 copies/PCR reaction

Lane 7: Samples with 1×10^7 pfu/ml, corresponding to 35,000 copies/PCR reaction

Lane 8: Samples with 1×10^8 pfu/ml, corresponding to 350,000 copies/PCR reaction

Lane 9: Samples with 1×10^9 pfu/ml, corresponding to 3,500,000 copies/PCR reaction

Lane 10: Samples with 1×10^{10} pfu/ml, corresponding to 35,000,000 copies/PCR reaction

Result: 35 copies of MS2 RNA could be detected with the kit

References

Fenollar, F. et al. (2003) *Antimicrob. Agents Chemother.*, **47**, 1665 – 1671

Vogelstein, B., & Gillespie, D., (1979) *Proc. Natl. Acad. Sci. USA* **76**, 615 – 619

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