


## High Pure Viral RNA Kit

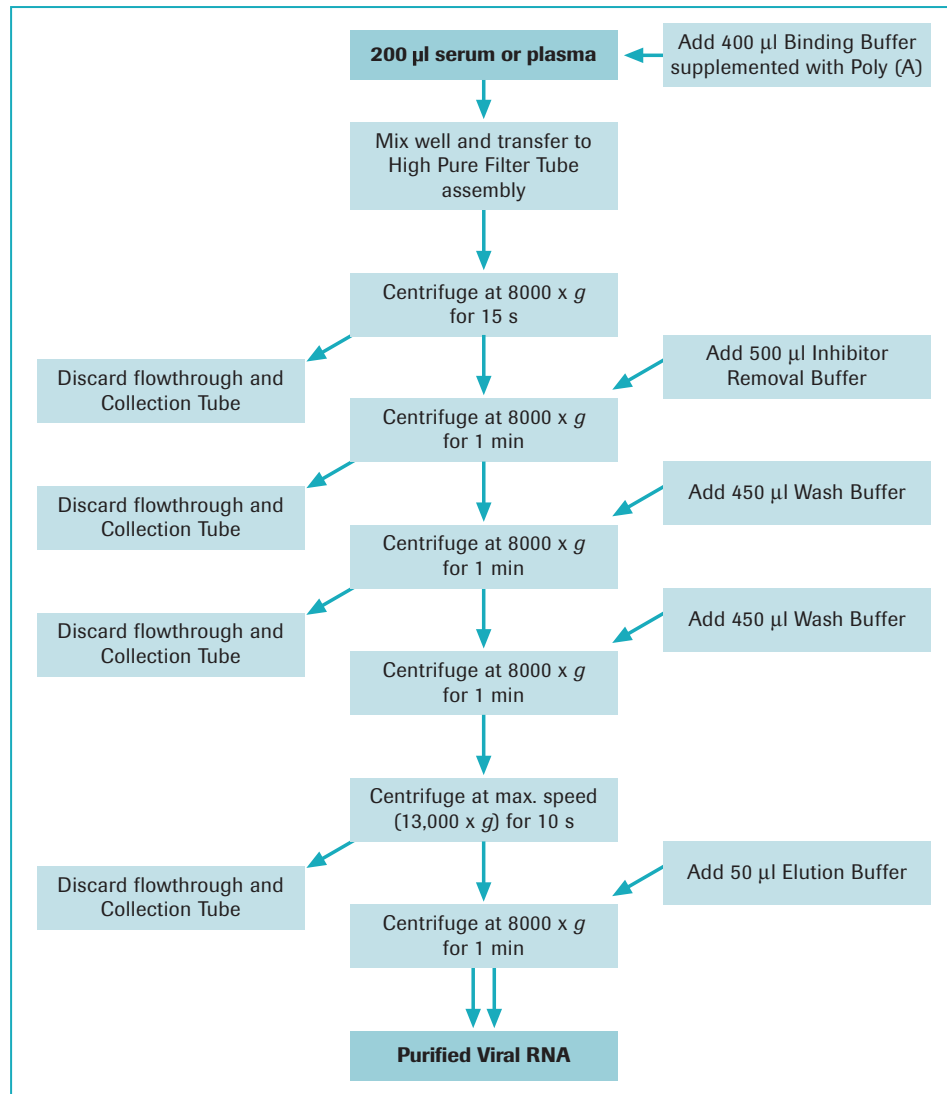
for isolation of RT-PCR templates from up to 100 samples

Cat. No. 11 858 882 001

<b>Principle</b>	Viruses, when lysed by detergent, release viral RNA. Then, in the presence of a chaotropic salt (guanidine HCl), viral RNA binds selectively to glass fiber fleece in a special centrifuge tube. The RNA remains bound while a series of rapid “wash-and-spin” steps remove contaminating cellular components. Finally, low salt elution removes the NA from the glass fiber fleece. The process does not require RNA precipitation, organic solvent extractions, or extensive handling of the RNA.
<b>Starting material</b>	<p>Research samples (200 – 600 µl) may be:</p> <ul style="list-style-type: none"> <li>● Serum</li> <li>● Plasma</li> <li>● Tears</li> <li>● Cell culture supernatant</li> <li>● Urine</li> <li>● Breast milk</li> </ul>
<b>Application</b>	<ul style="list-style-type: none"> <li>● Preparation of intact viral genomic RNA, which may be used directly as templates for RT-PCR</li> </ul> <p> <i>RNA preparations obtained are suitable for RT-PCR; they are not tested for other applications</i></p>
<b>Time required</b>	<ul style="list-style-type: none"> <li>● Total time: approx. 10 min</li> <li>● Hands-on time: &lt;10 min</li> </ul>
<b>Results</b>	<ul style="list-style-type: none"> <li>● Yield: 50 µl eluate is enough for 8 – 14 RT-PCRs</li> <li>● Purity: Purified RNA is free of intact virus, nucleases, and all cellular components that interfere with RT-PCR</li> </ul>
<b>Benefits</b>	<ul style="list-style-type: none"> <li>● <b>Saves time</b>, because the kit can prepare multiple RT-PCR templates in just minutes</li> <li>● <b>Accommodates a wide variety of samples</b>, because the same kit can purify viral RNA from several bodily fluids</li> <li>● <b>Minimizes RNA loss</b>, because the kit removes contaminants without time-consuming precipitation or solvent extraction</li> <li>● <b>Increases lab safety</b>, because the kit minimizes the handling of potentially hazardous samples and does not use hazardous organic solvents</li> </ul>

## How to use the kit

### I. Flow diagram



### II. Kit contents

- Binding Buffer containing guanidine HCl and Triton X-100 (2 x 25 ml)
- Carrier RNA, Poly(A), lyophilized (2 mg)
- Inhibitor Removal Buffer (33 ml)
  - ⚠ Add 20 ml absolute ethanol to buffer before use.
- Wash Buffer (2 x 10 ml)
  - ⚠ Add 40 ml absolute ethanol to each Wash Buffer before use.
- Elution Buffer (30 ml)
- High Pure Filter Tubes (100 tubes)
- Collection Tubes, 2 ml (400 tubes)

### III. Additional materials needed

- Absolute ethanol
- Standard tabletop microcentrifuge capable of a 13,000 x g centrifugal force
- Microcentrifuge tubes, 1.5 ml, nuclease-free

### IV. Protocol for preparing RNA from 200 µl samples



See the package insert supplied with the kit for instructions on processing 200 – 600 µl samples.

- 1 To a nuclease-free 1.5 ml microcentrifuge tube:
  - ▶ Add 200 µl serum or plasma.
  - ▶ Add 400 µl working solution [Carrier RNA-supplemented Binding Buffer] and mix well.

*The RNA yield can be increased two-fold with an optional incubation step, thus resulting in higher sensitivity. After adding the Binding Buffer to the sample, simply incubate the mixture at 15 to 25°C for 10 min. This incubation step can be omitted when time to result is critical.*
- 2 To transfer the sample to a High Pure Tube:
  - ▶ Insert one High Pure Filter Tube into one Collection Tube.
  - ▶ Pipette entire sample into upper buffer reservoir of the Filter Tube.
- 3 Insert the entire High Pure Tube assembly into a standard tabletop microcentrifuge, then centrifuge the tube assembly for 15 s at approx. 8000 x g.
- 4 After centrifugation:
  - ▶ Remove the Filter Tube from the Collection Tube and discard the Collection Tube.
  - ▶ Insert the Filter Tube into a new Collection Tube.
- 5 After reinserting the Filter Tube:
  - ▶ Add 500 µl Inhibitor Removal Buffer to the upper reservoir of the Filter Tube assembly and centrifuge 1 min at 8000 x g.
  - ▶ Discard flowthrough and combine Filter Tube with a new Collection Tube.
- 6 After removal of inhibitors:
  - ▶ Add 450 µl Wash Buffer to the upper reservoir of the Filter Tube.
  - ▶ Repeat the centrifugation (as in step 3).
- 7 After the first wash and centrifugation:
  - ▶ Repeat the discard step (Step 4).
  - ▶ Repeat the wash step (Step 6).
  - ▶ Leave the Filter Tube-Collection Tube assembly in the centrifuge and spin it for 10 s at maximum speed (approx. 13,000 x g) to remove residual Wash Buffer.
- 8 Discard the Collection Tube and insert the Filter Tube in a clean, nuclease-free 1.5 ml microcentrifuge tube.
- 9 To elute the viral RNA:
  - ▶ Add 50 µl of Elution Buffer to the upper reservoir of the Filter Tube.
  - ▶ Centrifuge the tube assembly for 1 min at 8000 x g.



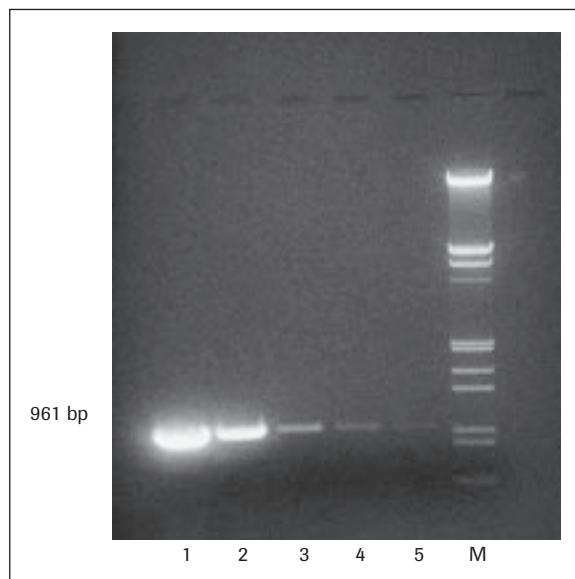
- 10 The microcentrifuge tube now contains the eluted viral RNA. You may:
  - ▶ EITHER use an aliquot of the eluted RNA directly in RT-PCR
    - ! Use 3.5 – 6  $\mu$ l of the eluate for the reverse transcriptase reaction.
  - ▶ OR store the eluted RNA for later analysis at  $-80^{\circ}\text{C}$ .

## 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 Viral RNA Kit, see page 105.

## Typical result with the kit

The kit was used to prepare genomic RNA from viruses [for example, hepatitis C virus (HCV), hepatitis G virus (HGV), and human immunodeficiency virus (HIV)] for research applications. Each preparation was used as a template in RT-PCR. All these templates produced highly specific PCR products in good yield.



**Figure 22: RT-PCR analysis of MS2 RNA isolated with the High Pure Viral RNA Kit.** Serial dilutions of purified MS2 RNA were applied to the Filter Tubes, washed and eluted following the kit protocol. 3.5  $\mu$ l of the 50  $\mu$ l eluate were analyzed by two step RT-PCR using primers that resulted in a fragment of 961 bp. The indicated numbers of molecules per PCR correspond to assumed quantitative recovery.

**Lane 1:**  $3.5 \times 10^7$  molecules/PCR;  
**Lane 2:**  $3.5 \times 10^5$  molecules/PCR;  
**Lane 3:**  $3.5 \times 10^3$  molecules/PCR  
**Lane 4:**  $3.5 \times 10^2$  molecules/PCR  
**Lane 5:** 35 molecules/PCR  
**Lane M:** DNA Molecular Weight Marker III

**Result:** Even with theoretically 35 molecules recovered, a detectable signal in agarose gel electrophoresis is obtained.

## References

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