Viral RNA extraction using Roche™ High Pure Viral RNA kit.

Created: Aug 21, 2008; **Last modified**: Jun 20, 2018, **Version:** 3.0

This protocol describes the method for isolating and purifying viral RNA from acellular biological specimens (plasma, serum, urine, or tissue culture supernatant) as described by the manufacturer (High Pure Viral RNA Kit, Roche, Dresden Germany, Catalogue # 11858882001). This kit is very recommended for routine and research use given its fast-processing speed (below 20 minutes from start to finish). This kit removes inhibitors that interfere with downstream assays, ensuring greater assay specificity, sensitivity, and reproducibility. It also has the advantage that it minimizes RNA loss as it omits steps that are normally included in other methods. In addition, it eliminates the use of hazardous organic compounds such as caesium chloride, phenol, chloroform, and the like. NOTE: The manufacturer recommends the use of Proteinase K for biospecimens having cells, please refer to the kit's manual for this purpose.

Procedure

1. To a sterile, nuclease free 1.5 mL Eppendorf microcentrifuge tube add 200 µL of plasma, serum, urine, or tissue culture supernatant).

2. Add 400 µL Binding Buffer and 40 µl of Poly A of carrier RNA solution to the same microcentrifuge tube bearing the sample, mix by pipetting with the same P1000 tip.

3. To maximize viral RNA yield, incubate this mix at room temperature for 10 minutes.

4. Insert one High Pure Filter Tube into one Collection Tube and transfer the entire biospecimen + binding buffer mix into the upper reservoir of the Filter Tube using the same P1000 pipet tip used above.

5. **SPIN 1:** Insert the entire High Pure Filter Tube assembly into a standard table-top centrifuge and spin for 1 min at 8,000 × g.

6. After centrifugation: Remove the Filter Tube from the Collection Tube; discard the flow-through and insert the Filter Tube into the Collection Tube (or a new one) and add 500 µL of Inhibitor Removal Buffer to the upper reservoir of the Filter.

7. **SPIN 2:** Insert the entire High Pure Filter Tube assembly into a standard table-top centrifuge and spin for 1 min at 8,000 × g.

8. After centrifugation: Remove the Filter Tube from the Collection Tube; discard the flow-through and insert the Filter Tube into the Collection Tube (or a new one) and add 450 µl of Wash Buffer to the upper reservoir of the Filter.

9. **SPIN 3:** Insert the entire High Pure Filter Tube assembly into a standard table-top centrifuge and spin for 1 min at 8,000 × g.

10. After centrifugation: Remove the Filter Tube from the Collection Tube; discard the flow-through and insert the Filter Tube into the Collection Tube (or a new one) and add 450 µl of Wash Buffer to the upper reservoir of the Filter.

11. **SPIN 4:** Insert the entire High Pure Filter Tube assembly into a standard table-top centrifuge and spin for 1 min at 8,000 × g.

12. After centrifugation: Discard the flow-through and re-insert entire assembly (WITHOUT REPLACING TUBES). Do not add further wash solution!

13. **SPIN 5:** Centrifuge for 10 seconds at maximum speed (approx. 13,000 × g) to remove any residual Wash Buffer.

14. After centrifugation: Discard the Collection Tube and insert the Filter Tube into a sterile, nuclease free 1.5 mL Eppendorf microcentrifuge tube and add 50 µl Elution Buffer to the upper reservoir of the Filter Tube.

15. **SPIN 6:** Insert the entire High Pure Filter Tube assembly into a standard table-top centrifuge and spin for 1 min at 8,000 × g.

16. The microcentrifuge tube contains the eluted, purified viral nucleic acids. Use the eluted nucleic acids directly in PCR (10 – 20 µl DNA eluate) or RT-PCR (3.5 µl viral RNA); or, store the eluted viral RNA at –80°C or the viral DNA at +2 to +8°C or –15 to –25°C for later analysis.

Notes

1. Poly-A supplement is resuspended in 400 µL of elution buffer. A 10% excess (40 µL) of the elution buffer is recommended by our local optimizations for the processing of 100 samples. Prepare eight 50 µL aliquots, each aliquot allowing for the processing of 12 samples. Keep them stored between -15 and -25ºC until further use and for no longer than 12 months.

2. Binding buffer can be prepared by adding 50 µL of poly A supplemental solution in 5 mL of the original buffer in the original bottle when processing several samples. However, the present protocol suggests preparing small amounts as mentioned in point 2 for the processing of small numbers of samples.

3. The inhibitor removal buffer is prepared by adding 20 mL of absolute ethanol and storing +15 to +25 °C). This solution should remain stable until the expiry date given by manufacturer for kit.

4. Wash buffer is prepared by adding 40 mL of absolute ethanol and storing at room temperature.

5. All buffer solutions should be clear and colourless, if precipitate is evident, heat at 37ºC until properly resuspended.

6. Samples should be processed on an ice tray for better RNA yields.

References

1. Roche GmbH; Dresden, DE. **High Pure Viral RNA kit.** Cat. No. 11 858 882 001. www.roche-applied-science.com

Revision history

1.0 Original document.

2.0 Optimized protocol.

3.0 Changes to document format only.