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User Bulletin

Viral RNA Extraction Miniprep System

For biological fluids containing RNA virus: serum, plasma, body fluids, and cell culture supernatant.

Downstream Application

- Northern, dot and slot blotting
- RT-PCR / Quantitative real-time PCR
- Poly A^+ RNA selection
- cDNA Synthesis/ Primer extension
- Array analysis
- *In vitro* translation

Product Contents

Cat. No	GVR1001	GVR1002
Preps	50	250
RXV Buffer	35ml	190ml
WS Buffer	15ml	45ml x 2
RNA Carrier	1	1
Proteinase K	10mg	3x10mg
RNase-free ddH_2O	1.5ml x 2	15ml
RNA Mini Column	50	250
Collection Tube	50	250
Protocol	1	1

All buffers need to be mixed well before use.

Shipping & Storage

Viogene Viral RNA Extraction System is shipped at ambient temperature and stored for up to 6 months.

If precipitation forms by freezing temperature on any buffer, warm up at 37℃ to redissolve.

Protocol

❖ **Please read the following notes before starting the procedure.**

- **WARNING**, strong acids and oxidants (for instance, bleach) should not be used together with RXV buffer (because this kind of reaction would produce toxic cyanide)!!!
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Important Notes

<Note>: Preheat RNase-free ddH_2O to 80°C.

- Add 1 ml sterile ddH_2O to each tube to reconstitute the provided Proteinase K by vortexing. Store the solution at 4 ℃.

-for GVR1001- Add 60 ml of ethanol (98-100%) to the WS Buffer bottle when first open the bottle.

-for GVR1002- Add 180 ml of ethanol (98-100%) to the WS Buffer bottle when first open the bottle.

Viogene's unique design — EasyLid™

The EasyLid™ is designed to prevent contamination during the procedure.

Tips for EasyLid™ –
Twist the arm of the cap and pull the cap to break the EasyLid™.

1. **Add RNA carrier to RXV Buffer.**
   Add 1 ml RXV Buffer to the RNA carrier tube, vortex to dissolve and transfer to the RXV Buffer bottle, store at 4°C.

2. **Pipet 150 µl sample (serum, plasma, body fluids, and cell culture supernatant) into a 1.5 ml tube.**

3. **Add 570 µl of carrier added RXV Buffer to the sample, mix by vortexing.**
   Through mixing is required for sample lysis. If the sample volume is larger than 150 µl, increase the amount of RXV buffer proportionally.

4. **Add 10 µl Proteinase K to the sample and incubate at 60°C for 60 minutes then place the sample in 80°C and incubate for 10 minutes to inactivate Proteinase K.**

5. **Add 570 µl of ethanol (98-100%) to the sample, and mix by vortexing.**
   If the starting sample is larger than 150 µl, increase the amount of ethanol proportionally.

6. **Place a RNA Column in a 2 ml Collection Tube, apply 650 µl of the ethanol added sample from step 5 to the RNA Column, close the cap, centrifuge at 6,000 x g (8,000 rpm) for 1 minute, and discard the filtrate.**
   If the solution remains above the membrane, centrifuge again at 13,000 rpm.

7. **Repeat step 6 for rest of the sample.**

8. **Wash the column twice with 500 µl of ethanol added WS Buffer by centrifuging at full speed (13,000 rpm or 10,000 x g) for 1 minute, and discard the filtrate.**
   Add 60 ml (for GVR1001) or 180ml (for GVR1002) of ethanol (98-100%) to the WS Buffer bottle when first open the bottle.

9. **Centrifuge at full speed for 5 minutes to remove traces of WS Buffer.**
   Residual ethanol may cause the low A_{260}/A_{230} and inhibit reverse transcriptase activity.

10. **Transfer the column to a RNase-free 1.5 ml tube (not provided), add 50 µl of preheated (80°C) RNase-free ddH_2O, and centrifuge at full speed for 1 minute to elute RNA.**

11. **Store RNA at -70°C.**