User Bulletin

VIOGENE
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VER.:30A

Viral RNA Extraction Miniprep System (Non Flu)

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
- NGS
- Gene editing

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 concentrate	10mg	3x10mg
RNase-free ddH_2O	6ml	27ml
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°C 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)!!!

Important Notes

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

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.

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 50°C for 10 minutes.

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 RNase-free ddH_2O, and centrifuge at full speed for 1 minute to elute RNA. Add another 50 µl of RNase-free ddH_2O, and centrifuge at full speed for 1 minute to elute RNA again, total of around 80µl after twice elutions.

11. Store eluted RNA at -70°C.