

Viral RNA Miniprep System

For Serum, Plasma, Body fluids, and Cell culture supernatant.

Kit contents:

RXV Buffer (1), WS Buffer (2), RNA carrier (1), RNase-free ddH₂O (1), Viral RNA column (250), Collection tube (250), 1.5 ml Elution tube (250) and protocol (1)

Protocol:

<Note>: Preheat RNase-free ddH₂O to 80°C.

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. Incubate the vortexed sample at room temperature for 10 minutes.

5. Add 570 µl of ethanol (96-100%) 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 Viral RNA column in a 2 ml Collection tube, apply 650 µl of the ethanol added sample from step 5 to the Viral 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 30 ml of ethanol (96-100%) to the WS Buffer bottle when first open the bottle.

9. Centrifuge at full speed for 3 minutes to remove traces of WS Buffer.

Residual ethanol may inhibit reverse transcriptase activity.

10. Transfer the column to a RNase-free 1.5 ml Elution tube, add 50 µl of preheated (80°C) RNase-free ddH₂O, and centrifuge at full speed for 1 minute to elute RNA.

11. Store RNA at -70°C.