

12. Remove the supernatant.
13. Dissolve pellet (*see Note 2*) in the storage buffer and centrifuge for 10 min at 30,000g.
14. Withdraw the supernatant (discard the pellet) and repeat **steps 11–14**.
15. Determine virus concentration by measuring UV light absorption at 260 nm (*see Note 3*).
16. Store the virus frozen at -70°C (*see Note 4*).

3.3. Extraction of Virion RNA (see Note 5).

1. Pipet out 200 μL purified virion preparation into an Eppendorf tube, add 25 μL 10% SDS (w/v), 25 μL 10X RNA extraction buffer, and 250 μL phenol.
2. Vortex for 20 s.
3. Centrifuge for 4 min at 14,000 rpm in an Eppendorf microcentrifuge.
4. Transfer the upper (aqueous) phase to a new tube, then add 125 μL phenol (*see Note 6*) and 125 μL chloroform (*see Note 7*).
5. Vortex for 20 s.
6. Centrifuge for 4 min at 14,000 rpm.
7. Transfer the upper phase to a new tube, then add 250 μL chloroform.
8. Vortex for 20 s.
9. Centrifuge for 3 min at 14,000 rpm.
10. Transfer the upper phase to a new tube and add 2.5 vol ethanol.
11. Keep on ice for 30 min.
12. Centrifuge for 12 min at 14,000 rpm.
13. Discard supernatant, wash the pellet by vortexing 30 s with 200 μL of 70% ethanol.
14. Centrifuge for 3 min at 14,000 rpm.
15. Discard the supernatant.
16. Dry the pellet in SpeedVac (under vacuum) for 3–5 min.
17. Dissolve in 50 μL DEPC-treated water.
18. Store the RNA preparation at -70°C (*see Note 8*).

3.4. Electrophoresis of Virion RNA

1. Melt the stored RNA preparation slowly on ice.
2. Vortex for 10 s.
3. Spin down all the droplets by centrifugation for 10 s at 14,000 rpm (e.g., in Eppendorf microcentrifuge).
4. While melting RNA samples, cast a 1% agarose gel in an autoclaved 0.5X TBE buffer. The electrophoresis tank should be pretreated with hot 1% SDS for 15 s, followed by immediate rinsing three times with sterile distilled water.
5. Load 0.1–0.5 μg RNA to each well in a sterile RNA loading solution.
6. Run electrophoresis for 1–3 h, depending on the length of the unit (the longer the unit, the better separation of the RNA segments) at 100 V.
7. Stain the gel with ethidium bromide (0.001% [w/v] solution) for one-half hour, then examine the gel under UV lamp.