3. Incubate filtrate in a 40°C water bath for 1 h (*see* **Note 24**).
4. Centrifuge for 20 min at 10,000*g* at 4°C and retain the supernatant.
5. Adjust supernatant to pH 8.0 with 1*M* NaOH. Measure initial extract. While stirring, add Triton X-100 to 1.5% (v/v) and sodium citrate powder to 0.01*M* final concentration.
6. Centrifuge for 10 min at 10,000*g* at 4°C, and retain the supernatant.
7. Centrifuge for 90 min at 100,000*g* at 4°C. Resuspend the pellet in buffer B (*see* **Note 25**).
8. Centrifuge for 10 min at 10,000*g* at 4°C, and retain the supernatant.
9. Layer supernatant over 0.2 vol (about 8 mL) 20% sucrose in buffer B and centrifuge at high speed, as in **step 8**.
10. Resuspend the pellet in buffer B, and centrifuge briefly at 8000–10,000*g* at 4°C.
11. Layer supernatant on a 10–40% sucrose density gradients prepare in buffer B, centrifuge, and collect the virus-containing fraction, as described previously (*see* **Notes 7–9**).
12. Dilute the sucrose-containing fractions with buffer B and centrifuge for 90 min at 100,000*g* at 4°C to concentrate virus. Resuspend the pellet in buffer B, which can be resuspended overnight at 4°C (*see* **Note 26**).

3.5. RNA Isolation from Virions

1. Virus in the same buffer used after the final purification step can be used here (*see* **Notes 27** and **28**).
2. Add SDS to 1% (v/v) final concentration (i.e., add 5 µL of a 20% stock solution for every 100 µL virus preparation).
3. Incubate at 55°C in a heat block or water bath for 5 min.
4. Add an equal volume of Tris-equilibrated phenol, preheated to 55°C.
5. Vortex vigorously for 5 s and centrifuge at 10,000*g* for 2 min.
6. Remove aqueous phase to a fresh (RNase-free) microcentrifuge tube. Add an equal volume of chloroform (chloroform:isoamyl alcohol [24:1]).
7. Vortex vigorously for 5 s and centrifuge at 10,000*g* for 1 min.
8. Remove aqueous phase to a fresh (RNase-free) microcentrifuge tube. Add 0.5 vol of 7.5*M* ammonium acetate. To this, add 2.5 vol of cold (–20°C) reagent grade ethanol.
9. Invert tubes until fully mixed. Place in the cold (–80°C for 30 min or –20°C for 1 h).
10. Centrifuge at 12,000*g* for 25 min at 4°C. Remove supernatant and save pellet. Add 1 vol (of original virus prep) of cold 70% ethanol (made with DEPC-treated water).
11. Immediately centrifuge at 12,000*g* for 5 min. Remove supernatant and save pellet. Vacuum-dry pellet (10–15 min without heat).
12. Resuspend in a small volume (ca. 20 µL of DEPC-treated water or RNase-free TE buffer).
13. Dilute a 5-µL aliquot in DEPC-treated water to obtain absorbances at 260 and 280 nm on an UV spectrophotometer to determine yield (20 OD_{260} = 1 mg RNA).