3. Filter the homogenate through cheesecloth and measure the volume of the filtrate.
4. Add 0.2 vol of a 2:1 mixture of chloroform:*n*-amyl alcohol to the filtrate for clarification. Homogenize at low speed for 30 s, transfer the homogenate to a beaker, and stir slowly at 4°C for 30–45 min.
5. Transfer the homogenate into centrifuge bottles and centrifuge at 10,400*g* in a Sorvall GSA Rotor for 10 min.
6. Carefully remove the upper phase and measure the volume. Discard the lower phase.
7. Transfer the upper phase into a large beaker and stir with a stir bar. While stirring briskly, dissolve PEG, average mol wt 8000, to 10% (w/v) and sodium chloride to 0.25*M*. Stir at reduced speed for 60 min at 4°C.
8. Transfer the solution into centrifuge bottles and centrifuge at 10,400*g* for 30 min.
9. Pour off and discard the supernatant. Resuspend each virus-containing pellet in 10 mL of 0.1*M* phosphate resuspension buffer, pH 7.0 (*see* **Note 3**). Store at 4°C overnight.

3.1.2. Day 2 (and Day 3 if required)

10. At the beginning of d 2 (or d 3; *see* **step 15**), remove the frozen sucrose gradients from the freezer and allow to thaw 1–2 h at room temperature. Once thawed, keep the tubes at 4°C (*see* **Note 4**).
11. Mix the resuspended virus-containing pellets by hand or on an agitator to thoroughly disperse.
12. Transfer the virus suspensions into tubes and centrifuge in a Sorvall SS34 rotor at 11,950*g* for 10 min.
13. Carefully remove supernatants and filter them through a tissue (Kleenex) to remove solids.
14. Form a sucrose pad by pipeting 7 mL of 30% sucrose (w/v) in phosphate resuspension buffer to a polycarbonate bottle with cap assembly to fit a Beckman 60Ti rotor. Gently layer 19.7 mL of the virus solution onto the sucrose cushion. Centrifuge at 252,000*g* for 2 h (*see* **Note 5**).
15. Pour off the supernatant and invert the bottles to drain. Resuspend each pellet in 1.0 mL of 0.1*M* phosphate resuspension buffer, pH 7.0. Agitate the pellets on a shaker for 2 h (2-d procedure), or agitate and leave overnight at 4°C (3-d procedure) for thorough resuspension.
16. Transfer the virus into tubes and centrifuge at 11,950*g* in a Sorvall SS34 rotor for 10 min. Carefully remove the supernatant (*see* **Note 6**).
17. Layer 1.0 mL of the virus solution onto each of the sucrose gradients. Centrifuge the virus in a Beckman SW41 rotor at 103,300*g* for 2 h (*see* **Note 7**).
18. Fractionate the gradients by displacement on an ISCO analyzer equipped with a UV monitor measuring absorbtion at 254 nm. Fractionation will require the preparation of a 60% sucrose (w/v) displacement solution. Virus will sediment near the middle of the tubes (*see* **Note 8**).
19. Combine the virus fractions and measure the concentration by u.v. absorbance assuming an extinction coefficient at 0.1% and 260 nm of E = 8.0.