20. Store the virus frozen in the sucrose solution from the gradient. The virus can also be pelleted by ultracentrifugation, resuspended in 0.1*M* phosphate resuspension buffer, pH 7.0, and stored frozen in the absence of sucrose, but significant losses may occur in this case.

3.2. Potato Leaf-Roll Virus Purification

3.2.1. Day 1

1. Using a Waring blender, grind leaf and stem tissue in 0.1*M* trisodium citrate, pH 6.0, containing 0.5% 2-mercaptoethanol (v/v) and 5% Celluclast (v/v), in a volume of 2 mL/g of tissue (*see* **Note 9**).
2. Stir the mixture at 27°C for 2 h or until the leaves are completely liquidized.
3. Adjust the pH of the solution to 7.0 by adding saturated Na_2HPO_4.
4. Add 0.33 vol each of chloroform and butan-1-ol (i.e., for every 900 mL of extract, add 300 mL each of chloroform and butan-1-ol). Stir for 30 min (*see* **Note 10**).
5. Centrifuge at 20°C in a Sorvall GSA rotor at 10,400*g* for 15 min (*see* **Note 11**). Retain the upper phase and discard the lower phase.
6. To the aqueous upper phase, add PEG 6000 to a final concentration of 8% (w/v) and NaCl to a final concentration of 0.2*M*.
7. Stir for 1 h at room temperature.
8. Centrifuge at 20°C in a Sorvall GSA rotor at 10,400*g* for 15 min.
9. Discard the supernatant and resuspend the pellet in 1 mL of 0.02*M* phosphate buffer containing 1% Triton X-100 (v/v) per 5 g of starting material. Store overnight at 4°C.

3.2.2. Day 2

10. Centrifuge the suspension at 20°C in a Sorvall GSA rotor at 16,300*g* for 10 min.
11. Transfer the supernatant and centrifuge through a layer of 20% sucrose (w/v) in 0.02*M* phosphate buffer, pH 7.5 (a sucrose cushion) at 302,000*g* in a Beckman 50.2 Ti rotor for 90 min at 20°C (*see* **Note 12**).
12. Discard the supernatant and resuspend the pellet in 1 mL of 0.02*M* phosphate buffer, pH 7.5, per 25 g starting material. Store at 4°C for several hours or overnight.
13. Repeat **steps 10–12**, resuspending the pellet in 1 mL of 0.02*M* phosphate buffer, pH 7.5, per 100 g starting material (*see* **Note 13**).
14. Repeat **steps 10–12**, resuspending the final pellet in 0.5 mL of 0.02*M* phosphate buffer, pH 7.5. Store at 4°C overnight, if necessary (*see* **Note 14**).
15. Centrifuge in a microcentrifuge at full speed (12,000*g*) for 10 min; transfer the supernatant to a fresh tube.
16. To calculate the concentration of virus, dilute in 0.02*M* phosphate buffer, pH 7.5, and measure the absorbance at 260 nm. An absorbance of 8.6 (in a 1-cm path length) is assumed to indicate a virus concentration of 1 mg/mL^{-1} (**ref. *7***; *see* **Note 15**).