

3. Blend 60 g of leaves for 1 min in 120 mL of cold ( $\sim 4^{\circ}\text{C}$ ) extraction buffer. Squeeze the brei through two layers of cheesecloth and centrifuge immediately at 4000g for 10 min in a low-speed fixed-angle rotor (in a Sorval GSA rotor).
4. While the centrifuge is running, make six discontinuous gradients in  $25 \times 89\text{-mm}$  centrifuge tubes for a Beckman SW28 rotor. Pipet 8 mL of 600 mg/mL sucrose/mL in maintenance buffer into each tube to form the bottom layer. Then, gently pipet 5 mL of 300 mg/mL sucrose over the bottom layer to make a distinct interface between the two layers (*see Subheading 2.4., Note 3*).
5. Adjust the supernatant recovered from the low-speed centrifugation to pH 7.5. Then, load 20-mL aliquots on the top of each of the six discontinuous gradients. Centrifuge the gradients for 60 min at  $4^{\circ}\text{C}$  at 110,000g in a Beckman SW 28 rotor to concentrate the virus between the 300 and 600 mg/mL sucrose layers (*see Subheading 2.4., Note 4*).
6. Prepare a Celite filter pad during the 60-min centrifuge run. For this purpose, suspend 17 g of Celite in about 100 mL of maintenance buffer. Pour the slurry onto a Whatman 3MM filter paper in a 10-cm Buchner funnel. Insert the funnel into a 1-L sidearm flask attached to a strong vacuum pump. Pull a vacuum and quickly release the vacuum just before the liquid reaches the top of the pad. Then, gently pour 100 mL of maintenance buffer over the pad and suck this through the pad under a vacuum. Again, release the vacuum just as the liquid reaches the top of the pad. The pad should be firm, with just a slightly wet sheen. Gently pipet about 5 mL of maintenance buffer over the pad and store upright, attached to the sidearm flask (*see Subheading 2.4., Note 5*).
7. Collect the green band between the 300 and 600 mg/mL sucrose layers from the tubes with a 15-gage or larger diameter bore needle, bent at a right angle near the tip, and attached to a 50-mL syringe. Dilute the green material (usually about 30 mL total) with an equal volume of maintenance buffer that had been allowed to equilibrate to  $4^{\circ}\text{C}$ . Stir 1 g of Celite into the suspension.
8. Gently suck the maintenance buffer through the Celite pad with a vacuum. Again, leave a slight sheen at the top of the pad. Swirl the plant material recovered from the sucrose interface and gently layer it over the Celite pad while pulling a stronger vacuum. Try not to disturb the surface of the pad. When the green slurry is about 5 mm above the pad, slowly begin to add maintenance buffer to the pad, and wash with 100 mL of the buffer. The filtrate should be a light-tan color and should exhibit light scattering when held up to a focused light source (*see Subheading 2.4., Note 6*).
9. Pour the filtrate into 30-mL tubes and centrifuge at 90,000g in a fixed-angle rotor in a Beckman Type 30 rotor for 30 min at  $4^{\circ}\text{C}$  to pellet the virus. Small light-tan to slightly green pellets about 5 mm in diameter will usually be visible. The color of these pellets is an excellent indicator of the final purity that can be expected. Quickly aspirate the solution from the pellets and resuspend them in a total volume of 1 mL of maintenance buffer.
10. Layer the suspension over a rate-zonal sucrose gradient formed 12–24 h previously by layering 5-, 10-, 10-, and 10-mL layers, respectively, of 50, 100, 200, and 300 mg of sucrose/mL of maintenance buffer.