Table 1
Preparation of Sucrose Solutions for 6–24% (w/v) Sucrose Gradients

	% (w/v) Sucrose solutions			
	6%	12%	18%	24%
60% (w/v) Sucrose	2.5 mL	10 mL	15 mL	20 mL
200 m*M* Potassium phosphate buffer, pH 6.8	12.5 mL	25 mL	25 mL	25 mL
20% (v/v) Triton X-100	125 µL	250 µL	250 µL	250 µL
H_2O	9.875 mL	14.75 mL	9.75 mL	4.75 mL

2. Materials

1. Borate buffer: 0.5*M* boric acid (H_3BO_3); bring to pH 9.0 with NaOH. Store at room temperature indefinitely. Chill the buffer to 4°C prior to virus purification.
2. 20% (v/v) Triton X-100: Add 20 mL of Triton X-100 to 80 mL of H_2O and autoclave for 5 min. When the solution cools to approx 60°C, swirl intermittently to prevent two phases from forming. Store at 4°C indefinitely.
3. Potassium phosphate buffer: Mix equal quantities of 200 m*M* solutions of KH_2PO_4 and K_2HPO_4 and store at 4°C. The pH of this solution should be 6.8. Dilute 200 m*M* potassium phosphate to obtain 50 m*M* and 10 m*M* potassium phosphate buffers and store at 4°C.
4. 60% (w/v) Sucrose: Dissolve 60 g sucrose to 100 mL with H_2O; store frozen or at 4°C.
5. 20% (w/v) Sucrose pad: Mix 30 mL of 60% (w/v) sucrose, 54 mL of 0.5*M* borate buffer, pH 9.0, 4.5 mL of 20% (v/v) Triton X-100 and 1.5 mL of H_2O.
6. 6–24% (w/v) Sucrose gradients: Prepare 24, 18, 12, and 6% (w/v) sucrose solutions (*see* **Table 1**).
7. Proteinase K (2 mg/mL) in H_2O. Store at –20°C.
8. Bentonite, prepared as described by Fraenkel-Conrat et al. ***(14)***: Stir 5 g of bentonite in 100 mL of H_2O, making sure that the bentonite is suspended well. Centrifuge at 600*g* for 10 min. Recover the supernatant and recentrifuge at 8000*g* for 20 min. Resuspend the pellet in 100 mL of 100 m*M* disodium ethylenediaminetetraacetate (EDTA), pH 8.0. Break the pellet up well with a glass rod and stir for 48 h at room temperature. Perform the two centrifuge steps as described previously. Resuspend the pellet obtained following the second centrifuge step in 100 mL of 10 m*M* sodium acetate, pH 6.0. Break the pellet up and stir overnight at room temperature. Centrifuge at 8000*g* for 20 min. Resuspend the pellet in 10 mL of 10 m*M* sodium acetate, pH 6.0. Determine the concentration of bentonite by adding 1.0 mL of bentonite to a preweighed weighing boat. Dry off the liquid in an oven at 60°C overnight and determine the weight of the bentonite. Dilute the bentonite to 10 mg/mL with 10 m*M* sodium acetate, pH 6.0. Store the bentonite stock at –20°C.