2. Freshly prepared saturated aqueous solution of Na_2HPO_4.
3. Chloroform. **Caution:** toxic, wear gloves, use and store in a fume cupboard.
4. Butan-1-ol. **Caution:** harmful by inhalation, wear gloves, store in a fume cupboard.
5. PEG 6000.
6. NaCl.
7. 0.02*M* Phosphate buffer, pH 7.5. Make a 0.02*M* solution each of Na_2HPO_4 and KH_2PO_4. Adjust to pH 7.5 by adding KH_2PO_4 to Na_2HPO_4 in a ratio of approx 1.5:8.5 (v/v), respectively. Store at 4°C.
8. Triton X-100.
9. 20% sucrose (w/v) in 0.02*M* phosphate buffer, pH 7.5. Make up fresh as required.
10. Waring blender.
11. Water bath at 27°C.

2.3. RNA

1. 10X RNA extraction buffer: 100 m*M* Tris-HCl, pH 7.6, 500 m*M* NaCl, 50 m*M* EDTA, 10% SDS (w/v). After sterilization, this buffer can be stored at room temperature for several months.
2. Water-saturated phenol containing *m*-cresol (9:1, v/v) and 0.1% 8-hydroxyquinoline (w/v). **Caution:** Phenol is toxic and can cause severe burns. *M*-cresol and 8-hydroxyquinoline are also toxic and harmful by inhalation. Wear gloves and work in a fume cupboard. To make a bulk quantity of this solution, mix 500 g of phenol in 140 mL water (phenol will dissolve more quickly if heated slightly in a water bath). Add 67 mL of *m*-cresol and 0.67 g of 8-hydroxyquinoline (8-OH) and dissolve. Keep the solution in the dark at 4°C.
3. Sterilized 3*M* sodium acetate, pH 6.5. The solution can be kept at room temperature for several months.
4. 100% Ethanol. Keep at 4°C.
5. Sterile, dH_2O. Keep at room temperature. All aqueous solutions and buffers used in procedures involving RNA should be autoclaved before use.

3. Methods

3.1. Barley Yellow Dwarf Virus Purification

3.1.1. Day 1

1. Begin with 500–1000 g of frozen tissue. Use 2–3 v/w of cold phosphate extraction buffer; all solutions throughout the procedure are kept at 4°C. Homogenize in a Waring heavy-duty blender at high speed. At the beginning of homogenization, gradually add frozen tissue to the buffer while blending. Once the entire sample has been added, blend at high speed for 30 s. Repeat this homogenization at approx 15-min intervals over a 3-h period. Keep the container cool on ice between steps (*see* **Note 1**).
2. Begin preparation of the sucrose gradients. Prepare a 20% (w/v) sucrose solution in 0.1*M* phosphate resuspension buffer, pH 7.0. Dispense 11 mL each into polyallomer tubes for a Beckman SW41 rotor. Freeze the tubes at –20°C (*see* **Note 2**).