2. Materials

1. 0.5*M* Phosphate buffer: Prepare a 0.5*M* solution of disodium hydrogen orthophosphate and adjust the pH to 7.2 with 0.5*M* potassium dihydrogen orthophosphate.
2. Virion extraction buffer: Add 1% (v/v) 2-mercaptoethanol to 0.5*M* phosphate buffer just before use (*see* **Note 1**).
3. Acid-washed sand.
4. Miracloth: purchased from Calbiochem (San Diego, CA) (*see* **Note 2**).
5. Butan-1-ol.
6. 20% (w/v) PEG (average mol wt 8000).
7. 10 m*M* phosphate buffer: Prepared by 50-fold dilution of 0.5*M* phosphate buffer described above.
8. 5*M* Sodium chloride.
9. 5X RNA extraction buffer: 0.5*M* sodium chloride, 5 m*M* ethylenediaminetetra-acetic acid disodium salt, 5% (w/v) sodium dodecyl sulfate, 0.1*M* Tris(hydroxymethyl)-methylamine (Tris), pH adjusted to 8.0 with hydrochloric acid.
10. Phenol:chloroform: Dissolve 250 g of molecular biology grade phenol in 250 mL of 0.1*M* Tris-Cl, pH 8.0, and add 1.25 g of 8-hydroxyquinoline. Equilibrate by extracting several times with 0.1*M* Tris-Cl, pH 8.0, and check that the pH is close to 8.0. Remove most of the overlying aqueous layer; add 240 mL of chloroform and 10 mL of isoamyl alcohol. Mix and allow phases to separate. Store refrigerated and protect from light.
11. Chloroform.
12. 3*M* sodium acetate, pH 5.0: Dissolve sodium acetate in water and adjust pH to 5.0 with glacial acetic acid. Adjust volume and treat with diethylpyrocarbonate (DEPC) to inactivate RNase. To each liter of solution add 1 mL of DEPC; mix and incubate overnight at room temperature in a loosely capped bottle. Autoclave to destroy residual DEPC.
13. Absolute ethanol.
14. Distilled water treated with DEPC, as described above.

3. Method

3.1. Virus Purification

1. Collect 20 g of systemically infected leaf tissue displaying infection symptoms (*see* **Notes 3** and **4**). Using a pestle and mortar, with a little acid-washed sand to aid homogenization, grind the leaf tissue in 60 mL of virion extraction buffer (*see* **Note 5**). Start grinding with a small amount of buffer and progressively add more. Continue grinding until the tissue is well-macerated.
2. Filter the homogenate through two layers of Miracloth into polypropylene centrifuge tubes. Squeeze as much liquid as possible out of material retained by the Miracloth without contaminating the filtrate with particulates.
3. Add butan-1-ol (0.8 mL/10 mL of filtrate) dropwise to the filtrate, while swirling the tube contents. Cap the tubes and incubate at room temperature for 15 min.