3. Resuspend the pellet by gentle shaking in 200 μL of 0.03*M* sodium phosphate, pH 8.0, per 100 g leaf tissue, and then centrifuge for 10 min at 12,000*g* in a cooled Eppendorf centrifuge. Using a Pasteur pipet, carefully transfer the virus-containing supernatant to a new 1.5-mL centrifuge tube. The preparation at this step, although containing still a little host-plant contamination, is good for viral RNA isolation and subsequent cDNA cloning. The quality can be controlled in an electron microscope and spectrophotometrically. The $OD_{260/280}$ of the preparation should range from 1.45 to 1.60. The concentration can be estimated from the extinction coefficient $E_{260\ nm} \approx 5.1–5.3$, which is equivalent to 1 mg/mL at 1 cm length of the light path.

3.5. Sucrose Gradient Centrifugation

If the preparation is to be used for antiserum production, a further purification step by sucrose density gradient centrifugation is recommended. Load at maximum 1 mL, with up to 3 mg of the virus suspension per each preformed sucrose gradient, ranging from 10 to 40% sucrose in 0.03*M* sodium phosphate, pH 8.0, and centrifuge for 4 h at 113,000*g* in a Beckman SW-28 rotor. Carefully collect the virus-containing zones from the gradients with an ISCO density gradient fractionator, according to the absorption profile, monitored at 254 nm. Dilute the virus-containing fractions four times in 0.03*M* sodium phosphate, pH 8.0, and sediment the virus by centrifugation at 123,000*g* for 2.5 h in a Beckman Ti-60 rotor. Resuspend the final pellet in 0.2 mL of the same buffer.

3.6. Storage of Purified Virus

For short periods, i.e., a few days, purified virus is stored at 4°C at the highest concentrations possible. For long-term storage, the purified virus should be aliquoted in useful amounts and stored frozen at –80°C or in liquid nitrogen.

3.7. Extraction of Viral RNA (see Note 11)

1. Mix the virus preparation with 1 vol of twofold proteinase K buffer and add proteinase K to an end concentration of 400 μg/mL. Incubate the mixture at 37°C for 30 min to digest the viral coat protein.
2. Add 1 vol of TE phenol to the digestion mixture and strongly vortex for 30 s; break the emulsion by a centrifugation for 4 min at room temperature and 8000*g* in a table centrifuge equipped with a swingout rotor. Using a pipet, carefully remove the upper aqueous phase, which contains the RNA.
3. Extract the aqueous phase again, first with phenol:chloroform mix, and then with the chloroform mix, as described above. Each time, carefully remove the aqueous phase.
4. Add 0.1 vol 3*M* sodium acetate, pH 5.2, and 2.5 vol of ice-cold 100% ethanol to aqueous phase and incubate for at least 30 min at –70°C. This will precipitate the RNA from the solution.