should be used in 38.5-mL tubes for the Beckman R50.2 Ti rotor. Later in the procedure, when the preparation has been concentrated into a smaller volume, 1–2 mL of sucrose can be used in 13.5-mL tubes for the Beckman R65 rotor, which can then be centrifuged at 368,000*g*. To load a sucrose cushion, place the sample in the tube first, leaving enough room to add the desired volume of sucrose solution. The sucrose is then underlayed by pouring it through a Pasteur pipet (or syringe), which has been placed in the tube so that the thin end is at the bottom. It is important to pour the sucrose solution through the pipet continuously so that air bubbles do not rise through the sucrose that is already in place and cause it to mix with the sample. Care must be taken not to disturb the sample once the sucrose cushion is in place.

13. For both PLRV and BYDV, the more thorough the purification procedure is, the smaller the final quantity of purified virus particles will be, i.e., each further round of differential centrifugation will result in the loss of virus particles because of incomplete sedimentation and general loss from manipulations. For many uses, e.g., RNA extractions, protoplast inoculations, and others, it is not necessary for the virus to be highly purified; partially purified particles are perfectly acceptable. This is especially important if a high yield of virus particles is needed. To obtain more virus particles from the purification procedure, the pellets from the slow-speed rounds of differential centrifugation can be resuspended and centrifuged again.
14. The pellet of virus particles produced by high speed centrifugation should ideally have a minimum of gelatinous sludge present. Dark pellets may result in false absorbance readings on the spectrophotometer, and excess gelatinous material affects the ability of the pellet to be completely resuspended. Care in the initial stages of the purification procedure, i.e., when removing the aqueous phase after the first centrifugation step, should prevent contaminants being present in the latter stages of the procedure.
15. If the final suspension of virus particles is not concentrated enough for the purpose for which it is required, it can be centrifuged at high speed once more and the pellet resuspended in a smaller volume. When measuring the absorbance of the virus suspension at 260 nm to estimate the virus concentration, it is advisable to check the absorbance at 280 nm. A high value at this wavelength indicates the presence of contaminating protein. Most of this can be removed by centrifuging the virus preparation through a sucrose gradient. An alternative to the method described in **Note 2** consists of layering 10, 20, 30, and 40% sucrose solutions into a centrifuge tube in a similar way to that described for the sucrose cushion. The lowest concentration of sucrose (i.e., 10%) is loaded first and, in ascending order, the other concentrations are underlayed, trying always to keep the flow of sucrose continuous. If the flow is interrupted, air bubbles will form and mix the different sucrose solutions. The gradients should be made up the day before use and stored at 4°C. This allows the sucrose to diffuse into a continuous concentration gradient. The sample is then very carefully layered on top of the gradient, trying to mix the interphase as little as possible.