5. Sediment the RNA by centrifugation for 20 min at 4°C and 12,000*g* in a cooled Eppendorf centrifuge.
6. Wash the resulting pellet once with 70% ethanol and centrifuge again for 5 min at 12,000*g* as above.
7. Dry the pellets for 5 min in a vacuum concentrator and resuspend it in 50 µL sterile water or TE buffer. The RNA preparation can be stored under 70% ethanol, as ethanol precipitate at –70°C, for many years. The quality and quantity of the RNA should be controlled spectrophotometrically for contaminating proteins (a ratio of absorbance 260/280 nm of ≥2.0 is indicative for the absence of protein) and by agarose gel electrophoresis, where the presence or absence of degradation can be observed.

4. Notes

1. The equipment list also contains branch names of some manufacturers and equipment. The authors do not want to state that the success of the experiments and methods relies on the use of these specific instruments. Instead, it is, of course, possible to use equivalent equipment and inventory. The procedures described here were developed for Prunus necrotic ringspot virus, but are also suitable for other ilarviruses (for suggestions for modifications, refer to subsequent notes).
2. The success in purification depends to a large degree on the greenhouse work, i.e., the choice and culture of the propagation plants. Only well-kept plants develop high titers of virus, and this makes purification easier. Second, it is highly advisable to plan the purification schedule carefully. Speed is a highly underestimated factor. Do not start with more plant material than you can process. The bottleneck is usually the first ultracentrifugation step, in which the volume is limited to about 450 mL when using one Ti-45 rotor.
3. Choice of propagation plants and their inoculation: The varieties of cucumbers or other herbaceous host plants may vary in their suitability for propagation; in case you start anew with work on ilarviruses, make sure you have a suitable variety available. The plants should be inoculated at an early developmental stage. For cucumbers, this is after the two cotyledones have developed and the first real leaf is barely visible. When inoculating with material from woody hosts, add PVP to your inoculation buffer.
4. Choice of extraction buffer: In some cases, the use of HEPES buffer for extraction and resuspending of the virus seems better than phosphate buffer. We have had this experience, especially with PDV, when 0.03*M* HEPES buffer, pH 7.5, worked best. In this case, acidify the clarified homogenate with 0.5*M* citric acid and neutralize back later with 0.5*M* NaOH.
5. Homogenization step: The homogenization should not last longer than 5 min; otherwise, it will disrupt virus particles. Addition of antioxidants 2-ME and DIECA stabilizes the virus particles during the first steps, but are obsolete later.
6. First purification steps: The yellowish supernatant should be decanted carefully without any green material, which lies between the supernatant and the unsoluble pellet. The green material interferes with the following steps.