2. Buffers for virus purification: 0.03*M* sodium phosphate, pH 8.0, is used as buffer for purified virus and for the homogenization step. For the homogenization step, add 0.02*M* 2-mercaptoethanol (2-ME) and 0.02*M* sodium DIECA just before use. **Caution:** 2-ME is harmful, handle it in a fume hood.
3. Chemicals for virus purification: Antifoam A solution (Sigma Chemie GmbH, Deisenhofen, FRG).
4. Triton X-100 solution.
5. 5*N* NaOH.
6. 40% (w/v) Sucrose solution in virus buffer, used as stock solution for preparation of sucrose gradients. Gradients are best prepared from four different sucrose concentrations: 10, 20, 30, and 40%. In order to prepare linear gradients, divide the nominal volume of the centrifuge tube, minus 1 mL, by four. This gives the necessary volume for each concentration per each gradient. Pipet the calculated amount of the 10% solution into the tube and underlay the remaining concentrations one after the other with a syringe and long cannula. The linear gradient is formed by diffusion during storage of the tubes overnight at 4°C.
7. TE buffer: 10 m*M* Tris-HCl, 1 m*M* ethylene diaminetetraacetic acid (EDTA), pH 7.5.
8. Twofold proteinase K buffer: 0.2*M* Tris-HCl, 0.3*M* NaCl, 25 m*M* EDTA, 2% (w/v) sodium dodecyl sulfate (SDS), pH 7.5.
9. TE phenol: Phenol saturated with TE buffer, pH of 7.5. **Caution:** Phenol and chloroform are dangerous for your health and the environment, as well as very corrosive. Handle them with gloves, protect your eyes with goggles, and work in a fume hood. Dispose of residues according to regulations. Remove spills, especially in centrifuges, and rotors immediately.
10. Phenol:chloroform mix: 50% (v/v) TE phenol, 48% (v/v) chloroform, and 2% isoamyl alcohol.
11. Chloroform mix: chloroform:isoamyl alcohol = 24:1 (v/v).
12. 3*M* Sodium acetate: Adjusted to pH 5.2 with acetic acid.
13. Proteinase K stock solution: 10 mg/mL in water. Store at –20°C.

3. Methods (*see* Note 1)

3.1. Propagation of Viruses

For most ilarviruses, cucumber *(Cucumis sativus) is* a good source of virus for propagation and purification. Most suitable are the varieties "Riesenschäl" and "Lemon"; however, if these varieties are not available, other varieties may be used. These should be tested before use for their sensitivity and the virus titer that is achieved upon inoculation. The best condition for cultivating cucumber plants is 24–25°C with an 18-h photoperiod. In uncontrolled conditions, the virus concentration in plants varies seasonally. The most suitable times are spring, autumn, and winter, but additional light is essential. The time required to reach a maximum concentration in cucumber cotyledons is normally 3–6 d after inoculation, but it may take up to 10 d under unfavorable